

ORGANIC
AND
TOXICOLOGICAL CHEMISTRY

ORGANIC AND TOXICOLOGICAL CHEMISTRY

FOR MEDICAL STUDENTS

By

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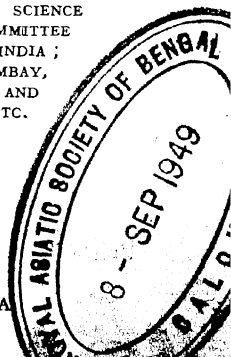
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PREFACE TO THE FIFTH EDITION

The fourth edition of the text had got out of print earlier than it was expected and the authors took this opportunity for a thorough revision of all the chapters and also for the introduction of some of the advances in the subject without increasing the bulk of the book. The emphasis laid on the varied applications of organic chemistry to biochemical problems will help the student to take an increasing interest in medical biochemistry and also to study the latter subject more intelligently. The special chapter on Vitamins containing numerous useful data on Indian foodstuffs will help to create an interest in the nutritional problems of India, and thirdly, the special chapters on Toxicological Chemistry would enable the student to study Pharmacology and Toxicology with profit in his later years of medical study. The text will therefore not only cater to the needs of an examinee in medical chemistry but also serve as a book of reference for his future use. Considering the otherwise high cost of medical education for an Indian student, the price of the book has been kept very low compared to the enormous increase in the cost of publication. It is, however, believed that with the varied informations incorporated in the text it would prove useful not only for the medical students but also for the B.Sc., B.Sc. (Tech.), B. Pharm. and other classes of students all over India. The authors desire to acknowledge with grateful thanks the useful suggestions and criticisms offered by many of their friends, particularly by Prof. R. Ganguly, M.Sc., R. G. Kar Medical College, Calcutta. They have been of inestimable value in revising the book for this edition.

Calcutta
The 15th August, 1946.

S. G.
K. N. B.

PREFACE TO THE FOURTH EDITION

The third edition of the book was out of print within a few months of its publication and the authors note with satisfaction the warm reception accorded to it in different parts of India. The difficulties about paper and press have delayed considerably the publication of this edition. The delay has, however, helped the authors to devote more time for its improvement by incorporating certain new and important subjects, for instance, the chemistry of penicillins, paludrine, technical D.D.T., etc. All the chapters have been carefully revised and many portions have been entirely rewritten and brought up to date as a result of which there has been an increase in the number of its pages. In the toxicological portion two new illustrations have been added and some interesting cases of poisoning by lead tetraethyl, hitherto unknown in India, have been described.

In the preparation of this edition, as also in the previous one, works of eminent toxicologists particularly of Webster, Modi and Sydney Smith, have been of inestimable value which the authors thankfully acknowledge. They also desire to record their grateful thanks to Dr. M. Swaminathan, D.Sc., of the All-India Institute of Hygiene & Public Health, Calcutta, for his valuable assistance in revising the chapter on Vitamins.

The book was originally written for medical students of Indian Universities but the manner in which the matters have been discussed and presented leads the authors to believe that it will prove useful to other students of science for whom a basic knowledge of organic chemistry is essential, such as those for B.Sc., B.Sc. (Tech.), B. Pharm., M.Sc. with pharmaceuticals, food and drugs, etc. Public Health students and students studying Biochemistry, Toxicology and Industrial Hygiene will also find the book useful, particularly its toxicological portions.

Calcutta
The 15th July, 1946.

S. G.
K. N. B.

PREFACE TO THE THIRD EDITION

In spite of numerous changes and considerable additions, the present volume may be regarded as the Third Edition of Ghosh & Boyd's Manual of Organic Chemistry for Medical Students, since the method of treatment and the arrangements of the subject matter dealt with in the Second Edition of that text have been generally left unaltered. As the second author (T. C. B.) of the previous editions had already retired from service and was about to leave India, when the publication of the Third Edition of the book was under consideration, the senior author felt it desirable to publish this volume, with his full approval, with an altered title and with the help of another joint author in his place.

Although the method of treatment and general arrangement remain practically the same as regards older matter, almost all the previous chapters have been carefully revised and rewritten and considerable new matter has been introduced. The criticism levelled against the previous editions as being too concise for medical students has been met by adding more details wherever it has been found desirable. More attention has been paid to the application of organic chemistry to biochemical problems, and also to synthetic drugs which are rapidly replacing those found to occur in nature. The rapid advance of our knowledge of the chemistry of vitamins and their wider application to medicine have encouraged us to attempt a detailed summary of this fascinating subject, and the chapter will we hope increase the usefulness of the book for a wider circle of readers.

Quite a new feature of this edition has been the addition of five chapters on Toxicological Chemistry. The inclusion of this subject has been actuated by the attention paid to it by the different Indian Universities in their medical and other curricula. The chapters incorporate in them in a nutshell the results of special study and wide practical experience of one of us in this important subject.

In the preparation of this edition, we have had to consult several standard books on organic and biochemistry and the physical data have been mainly drawn from Beilstein. The

large text books on toxicology which have been consulted freely are those of Witthaus, Autenrieth, Webster, J. P. Modi and Sydney Smith. We have also to thank Messrs. Butterworth & Co. (India) Ltd., for kindly providing us with six blocks printed in Modi's Text Book of Medical Jurisprudence and Toxicology.

In conclusion, the authors hope that the book will prove useful not only to medical students for whom it has been primarily written but also to pharmaceutical and public health students as well as to scientific workers interested in the application of organic chemistry to biochemistry, toxicology and medicine.

Calcutta
The 1st October, 1944.

S. G.
K. N. B.

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ORGANIC AND TOXICOLOGICAL CHEMISTRY

PART I

CHAPTER I.

GENERAL

Historical Introduction.—About the end of the 17th century, Léméry introduced the name Organic Chemistry in scientific literature. It included the chemistry of substances occurring in the vegetable and animal kingdoms as distinguished from substances occurring in the mineral world which were placed under Inorganic Chemistry. By the end of the 18th century, Lavoisier demonstrated the true nature of organic compounds and showed that they contained carbon, hydrogen and oxygen. The organic compounds were, however, believed to be produced in living organisms only through the agency of a mysterious force known as the *vital force*. In 1828, Wöhler synthesised *urea*, a characteristic product of animal metabolism, from cyanic acid and ammonia and thus broke the barrier between inorganic and organic compounds. The distinction is, however, still retained for reasons of convenience.

Definition of Organic Chemistry and Peculiarity of Organic Compounds.—Organic Chemistry is now defined as the *chemistry of carbon compounds*, and only the very simple compounds like carbon monoxide, carbon dioxide, metallic carbides, etc., are treated under Inorganic Chemistry.

The formation of innumerable organic compounds containing only a limited number of elements depends partly upon the *tétravalency of the carbon atom* but chiefly upon the *power of the carbon atom to link with itself* and with other elements forming long and stable compounds with open chains or rings. In Inorganic Chemistry the number of elements is large but the compounds formed by any one element is small owing probably to the lack of affinity between similar elements.

The majority of *naturally occurring* organic compounds consist of C and H (*e.g.*, methane, benzene, etc.) or of C, H and O (*e.g.*, alcohols, acids, sugars, etc.). A relatively smaller number of compounds consist of C, H and N or of C, H, N and O (*e.g.*, purines, amines, alkaloids, etc.) and a still fewer number contain S and P (*e.g.*, proteins, etc.). Iodine is found in thyroxine, one of the active principles of the thyroid gland. Of the metals, Fe is found in organic combination in hæmoglobin, the red pigment of the blood of all vertebrates, and Mg is an essential constituent of chlorophyll, the green pigment of plants. Some crustaceans and molluscs contain Cu instead of Fe in their blood pigment.

A very large number of carbon compounds containing non-metals like Cl, Br, I, N, S, etc., and a large number of organo-metallic compounds containing Hg, Pb, Zn, Mg, As, Sb, etc., have, however, been *prepared synthetically* in the laboratory and have added to the ever increasing number of organic compounds.

Recognition of Organic Compounds.—Organic compounds are readily distinguished from inorganic compounds by their *combustibility*. When a solid organic compound is gradually heated it will *char* and finally burn in contact with air without leaving any ash. Charring may not, however, be observed in certain cases where the substances *volatilize*, or in case the molecule contains enough oxygen to produce CO_2 and H_2O , *e.g.*, oxalic acid. The *evolution of CO_2* on oxidation is, of course, a more definite test for the presence of an organic compound. For this the organic compound, if solid or a non-volatile liquid, is heated with powdered cupric oxide CuO in a test tube and the CO_2 evolved is led into a solution of baryta or lime-water. In the case of a volatile liquid or gas the vapour is led over a glowing oxide of copper and the CO_2 evolved is tested as before. The presence of *inorganic matter* (non-volatile) in an organic compound is best observed by heating the substance carefully on a platinum foil, when the organic matter will burn away leaving the inorganic portion as ash.

Purification of a Solid by Crystallization.—An organic solid is usually purified by crystallization from a solvent.

The solvents most frequently employed are *organic liquids* like ethyl alcohol, methyl alcohol, acetone, chloroform, ether, petroleum ether, benzene, etc., although *water* is also found to be suitable in many cases. The advantage of organic solvents is that they generally boil at a lower temperature than water and hence not only do they cause less decomposition but they can also be easily removed by evaporation.

It is found that *a solvent will dissolve a compound which has a constitution similar to itself*. Thus, ethyl alcohol, which has got a hydroxyl group, will usually dissolve hydroxy compounds, and benzene, a hydrocarbon, will dissolve hydrocarbons more readily, and so on. There are, however, many exceptions to this rule, and an experimental trial with the solvent is often of more value.

The selection of a proper solvent is very important in crystallization, and the most suitable solvent is one in which the substance is soluble with difficulty in the cold but dissolves easily on warming. Sometimes a *mixture of solvents* is found better than a single pure solvent, and one solvent is selected in which the substance is easily soluble and another in which it is soluble with difficulty. Thus, mixtures of alcohol and water, alcohol and ether, chloroform and petroleum ether, are often found to be useful.

For crystallization, the substance is dissolved in a small amount of the hot or boiling solvent and filtered while still hot. If the substance is coloured, a little finely powdered *animal charcoal* (blood or bone charcoal) is also added before boiling in order to adsorb the colouring matter. On cooling the filtrate, crystals of the dissolved substance, which is less soluble in the cold solvent, are precipitated and are separated by filtration. On concentrating the filtrate, or *mother liquor* as it is called, a second crop of crystals appears and further crops of crystals are obtained by repeating the process. This process of purification is known as *fractional crystallization*, and it depends upon the fact that the less soluble substance crystallizes out first, then comes the more soluble, and so on. The crystals obtained in each fraction may be further purified by recrystallization from the same solvents.

Sublimation.—This is a process by which a solid substance is converted by heat directly into vapour and condenses back into a solid on cooling without passing through the liquid phase. Certain volatile solid substances (e.g., camphor, naphthalene, benzoic acid, etc.) can be

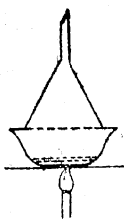


FIG. 1.

purified by this process from non-volatile impurities. A simple arrangement for sublimation consists of a small porcelain basin over which is placed a circular asbestos board pierced by a number of small holes (Fig. 1). A glass funnel is inverted over the asbestos board and the material to be sublimed is placed in the basin. The basin is then gently heated over a sand bath when the material vaporizes and condenses as fine crystals on the inner side of the cold funnel and can be easily scraped off.

Criteria of Purity of Solids.—A solid substance is considered pure only when it *melts sharply* at a definite temperature. When impure, the substance first begins to soften and there is usually a difference of several degrees between the softening point and the temperature at which it melts completely.

It may happen that two entirely different substances have nearly the same melting point. In such a case a mixture of the two will show a lower melting point whereas a mixture of two pure samples of the same substance does not show any such lowering: This method of determining the *mixed melting point* is sometimes used to establish the identity of an organic substance.

Determination of Melting Point.—The dry solid substance is finely powdered and taken in a thin-walled capillary tube closed at one end and measuring about 1 mm. in diameter and about 8 cm. long. The capillary tube is attached to a thermometer by means of a thin rubber band so that the substance is close to the middle of the bulb of the thermometer. A high boiling liquid, such as concentrated sulphuric acid, is taken in a long-

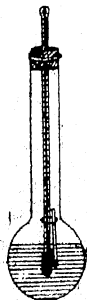


FIG. 2.

necked hard glass flask fitted with a cork having a shallow vertical groove on its side to allow for the escape of gases. The thermometer with the capillary tube passes through the centre of the cork and dips into the liquid (Fig. 2). The flask is placed over a wire gauze and gently heated from below. The temperature at which the substance melts to a transparent liquid is observed and taken as the melting point of the solid.

Purification of Liquids by Distillation.—An organic liquid is generally purified by distillation. The substance is taken in a distillation flask provided with a side-tube. The neck of the flask is fitted with a cork through which a thermometer is passed, the bulb of the thermometer reaching slightly below the level of the side-tube. The

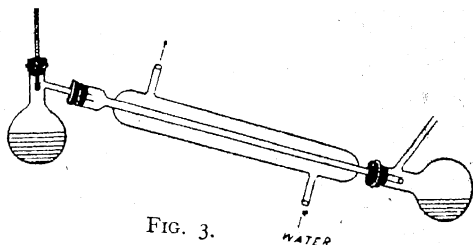


FIG. 3.

flask which is attached to a *condenser* is gently heated from below after adding a few pieces of unglazed porcelain to the liquid to prevent bumping and the temperature at which the liquid begins to boil is observed. The vapour of the liquid condenses and the distillate is collected in a *receiver* placed at the lower end of the condenser (Fig. 3).

For high-boiling liquids (boiling above 130°C), an *air-condenser* (i.e., a straight glass tube without any jacket) is used. For low-boiling liquids, a *water-cooled condenser* (e.g., Liebig's condenser, as in Fig. 3) is used, and in case the room temperature is high, a condenser cooled with *ice-water* may be necessary.

In the case of high-boiling liquids the flask is heated over a *wire gauze* or a *sand bath*. For liquids boiling below 80°C and especially when the liquid is inflammable, the flask is heated over a *water bath* or still better over a *steam bath*.

Fractional Distillation.—If the liquid to be distilled is pure it will boil over completely at a constant temperature.

If, however, it is a mixture the boiling point will gradually rise, the more volatile liquid coming over at a lower temperature. A separation of the different liquids of a mixture can thus be made by changing the receiver after the liquid boiling within a definite range of temperature (say, every 5°C) has collected. Each fraction collected in this way is refractionated until liquids of constant boiling point are obtained. This process of separation of different liquids in a mixture is known as fractional distillation. For a more efficient separation of liquids a *fractionating column* (e.g., a pear-shaped still head or better, a glass column packed with small pieces

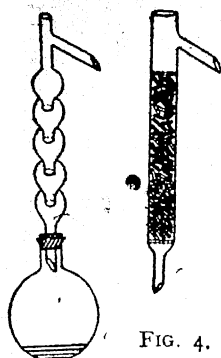


FIG. 4.

of narrow glass tubings) is fitted on the neck of an ordinary round-bottomed flask in place of the usual distillation flask with the side-tube (Fig. 4).

Vacuum Distillation or Distillation under Reduced Pressure.—When a liquid has a very high boiling point or

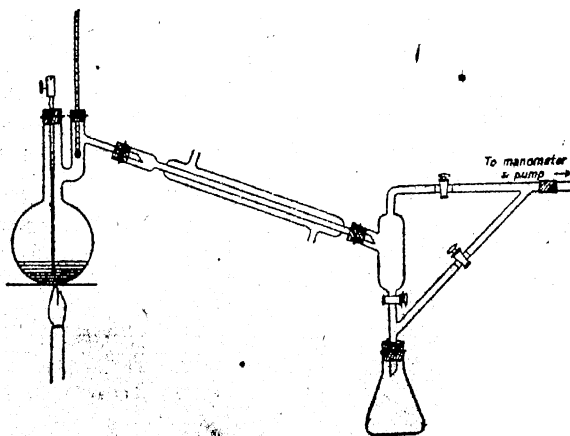


FIG. 5.

When it decomposes at its boiling point, it has to be distilled

in vacuo, i.e., at a reduced pressure. For vacuum distillation the ordinary arrangement for distillation can be made (as in Fig. 3, p. 5) but all the connections are made airtight and the receiver is connected by a side-tube with a manometer and with an efficient pump to create vacuum. Since the boiling point of a liquid diminishes with the fall of the pressure above it, the liquid will boil at a lower temperature the higher the vacuum and hence a decomposition is avoided.

For *fractional distillation in vacuo* a special distillation flask, such as a Claisen Flask, is used and special receivers (e.g., those by Brühl, Perkin, etc.) are selected so that the different fractions can be collected in separate vessels without releasing the vacuum (Fig. 5).

Steam Distillation.—This is a method of separating organic substances insoluble in water from non-volatile impurities whether solid or liquid. The method applies to those compounds which have an appreciable vapour tension at about 100°C , the boiling point of water. Some high-boiling liquids, such as aniline or chlorobenzene can be purified by this means, and all the *essential oils* which occur in various plants are isolated by steam distillation.

A current of steam from a small boiler is passed through the substance placed in a round-bottom flask (Fig. 6). The

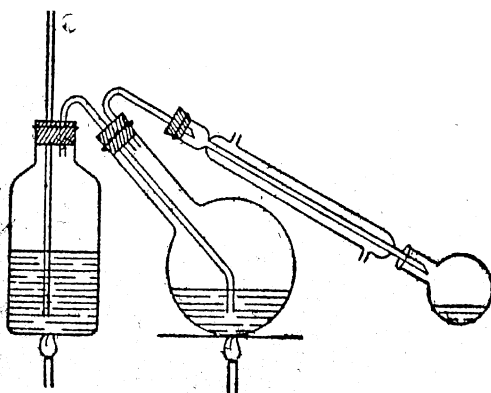


FIG. 6.

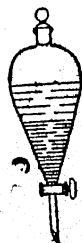


FIG. 7.

flask is set in a sloping position so that the contents do not

splash into the condenser and it is heated over a sand bath to prevent the condensation of much steam. The vapour of the substance condenses along with the steam in a water condenser and collects in the receiver. The distillate is transferred to a *separating funnel* (Fig. 7) in which the liquid which is immiscible with water gradually forms a separate layer and can be easily removed. The portion of the liquid which forms an emulsion with the water can then be dissolved out by shaking with a solvent like ether.

Boiling Point of a Liquid.—The boiling point of a liquid is determined in the same type of apparatus as that described for distillation (Fig. 3), only a smaller distillation flask is used if the amount of liquid is small.

When the liquid available is very small (say, a few drops) a *semi macro method* is employed. The liquid is taken in a narrow test tube which is attached to a thermometer by means of a rubber band. Into the liquid is dipped the open end of a sealed capillary tube (a melting point tube) and the thermometer with the test tube is then placed in the bath, used for the determination of melting point, which is heated very slowly (Fig. 8). The temperature at which a regular stream of bubbles comes out of the tube is taken as the boiling point of the liquid. The air in the capillary tube prevents the superheating of the liquid by supplying a slow stream of air bubbles in the beginning at the bottom of the liquid. This is followed by a regular stream of bubbles of the vapour when the boiling point of the liquid is reached. The mean of three readings is taken as the boiling point.

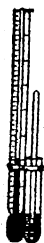


FIG. 8.

The boiling point of a liquid varies considerably with the atmospheric pressure above the liquid and it is, therefore, customary to mention the pressure when stating its boiling point, especially when it is distilled under reduced pressure (e.g., $120^{\circ}/10$ mm.). Ordinarily however, the pressure is understood to be the normal atmospheric pressure, i.e., 760 mm. of mercury, and no mention is made about this.

Criteria of Purity of Liquids.—A liquid is considered to be pure when it shows a constant and definite boiling point when distilled under a definite pressure.

Mixtures of a few liquids sometimes show a constant boiling point. For instance, a mixture of ethyl alcohol and water containing about 95.6 per cent of the alcohol will boil at a constant temperature of 78.1° at 760 mm. and it is almost impossible to concentrate the alcohol further by distillation. Similar *constant-boiling mixtures* (also known as *azeotropic mixtures*) are formed by methyl alcohol and acetone, acetone and chloroform, methyl alcohol and benzene, pyridine and water, and so on. Such a mixture is distinguished from a pure liquid by redistilling under a different pressure. A pure liquid will change its boiling point but the composition of the distillate will remain the same, whereas in the case of a constant-boiling mixture there will be a change both in boiling point and in the composition of the distillate.

CHAPTER II

QUALITATIVE AND QUANTITATIVE ANALYSIS

A. Detection of the Elements.

Carbon and Hydrogen.—A small amount of the dry powdered substance is intimately mixed with some freshly ignited, dry copper oxide powder and carefully heated in a dry, narrow, hard-glass test tube fitted with a cork and a bent glass tube. The *carbon dioxide* evolved is led into a clear solution of lime water kept in another test tube. The solution becomes turbid owing to the formation of calcium carbonate. The simultaneous formation of *water vapour* and its condensation on the upper and cooler parts of the test tube in which the mixture is heated proves the presence of hydrogen in the substance. A pinch of colourless anhydrous copper sulphate if sprinkled on the moisture is turned blue.

Oxygen.—There is no direct method of detecting the presence of oxygen, except when *water* is formed by heating the dry substance in an atmosphere of nitrogen. It is found, however, by a quantitative analysis which not only detects but also determines its quantity.

Nitrogen.—(i) A simple means of detecting the presence of nitrogen is to heat the substance with *soda-lime* (an intimate mixture of NaOH and CaO) in a hard-glass test tube and the ammonia evolved is recognized by its usual tests. The method, however, fails in the case of many compounds (e.g., amines, nitro-compounds, etc.) and it is, therefore, better to use the method of *sodium fusion* described below. (ii) *Lassaigne's Sodium Test*. This test serves for the simultaneous detection of nitrogen, halogens and sulphur. A pellet of *metallic sodium* about the size of a pea is heated in a narrow dry test tube until it melts and a pinch of the dry substance to be tested is slowly dropped into the melted sodium. The mixture is heated to redness until the decomposition is complete and the red-hot portion

of the test tube is dipped into distilled water kept in a mortar. It breaks and the contents of the tube are ground to a powder. The extract is filtered and the *aqueous filtrate* is divided into *three* portions for testing the elements mentioned above. To test for *nitrogen* the aqueous filtrate is treated with a few drops of a freshly prepared solution of ferrous sulphate, heated to boiling, cooled and a few drops of ferric chloride solution are added. It is then acidified with concentrated hydrochloric acid in order to redissolve the iron hydroxides. A blue precipitate or a bluish green solution due to the formation of Prussian Blue shows the presence of nitrogen in the original substance. The sodium fusion converts the nitrogen into sodium cyanide NaCN which is changed to sodium ferro-cyanide $\text{Na}_4\text{Fe}(\text{CN})_6$ on boiling with ferrous sulphate. The ferric chloride then converts the ferrocyanide into Prussian Blue $\text{Fe}_4[\text{Fe}(\text{CN})_6]_3$.

Halogens.—(i) *Beilstein Test.* A small piece of fine copper gauze is rolled at one end of a stout copper wire, the other end being pushed through a cork. The gauze is held in the non-luminous flame of a gas burner until the flame is colourless. The hot copper gauze is then dipped into a small quantity of the powdered organic compound and placed again in the non-luminous flame. A green colour indicates the presence of a halogen (Cl, Br, I). The copper oxide formed by heating the copper gauze decomposes the organic compound forming a volatile copper halide which imparts the green colour to the flame. The method is, however, not applicable in the presence of free acids, and some halogen-free substances such as urea or thiouræa, and some pyridine compounds also give this green flame.

(ii) *Lassaigne's Test.*—The *aqueous filtrate* obtained after the *sodium fusion* mentioned above contains the *sodium halide* and is acidified and heated with dilute nitric acid which decomposes any NaCN and removes the HCN . A white or yellow precipitate on the addition of a solution of silver nitrate will indicate the presence of a halogen. To find out the *nature of the halogen*, the precipitated silver halide may be treated with *ammonia* in which AgCl is easily

soluble whereas AgBr is soluble with difficulty and AgI is insoluble. The aqueous filtrate may also be treated with dilute sulphuric acid until it is just acid to litmus. A little carbon disulphide and chlorine water are added and shaken. A brown colour imparted to the carbon disulphide will indicate the presence of Br , a violet colour will indicate the presence of I , and the absence of any colour will indicate the presence of chlorine.

In case the substance also contains N or S , the aqueous filtrate should be acidified with dilute nitric acid and boiled in order to remove the HCN or H_2S which would otherwise give a precipitate with AgNO_3 .

Sulphur.—(i) The aqueous filtrate from sodium fusion which contains sodium sulphide is treated with a freshly prepared aqueous solution of sodium nitroprusside, $\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}] \cdot 2\text{H}_2\text{O}$, and a bright purple colour will indicate the presence of S . (ii) The aqueous filtrate may also be acidified with acetic and treated with an aqueous solution of lead acetate. A black or brown precipitate or colouration due to PbS will indicate the presence of S . (iii) If the substance contains both S and N , the aqueous filtrate will contain sodium thiocyanate NaCNS . The solution is neutralized with dilute HCl until it is faintly acid and treated with 2 or 3 drops of ferric chloride; there will be a blood-red colour due to the formation of ferric thiocyanate $\text{Fe}(\text{CNS})_3$. (iv) The original substance is fused with a mixture of Na_2CO_3 and KNO_3 and the melted mass is extracted with water and filtered. The S is oxidized to a sulphate and is tested by a solution of BaCl_2 after acidifying the extract with HCl or HNO_3 .

Phosphorus.—(i) The substance is heated with a mixture of concentrated sulphuric and nitric acids in order to oxidize the P into phosphoric acid. When the solution becomes colourless, it is cooled, diluted with water, and boiled with some ammonium nitrate. The solution is cooled, treated with a solution of ammonium molybdate and warmed, when a yellow precipitate of ammonium phosphomolybdate will be formed. (ii) The substance is fused with a mixture of Na_2CO_3 and KNO_3 and the melted mass is

extracted with water and filtered. The extract is acidified with nitric acid and treated with a solution of ammonium molybdate and warmed as before.

Arsenic.—Many organo-arsenic compounds are now used in medicine and various arsenic compounds, both organic and inorganic, have occasioned cases of poisoning, accidental or criminal. It is, therefore, important for medicolegal workers to be familiar with the methods used for the detection and estimation of As in presence of organic matter.

The methods for the detection and quantitative estimation of As have been described later in detail (see pp. 551-567).

Antimony.—Many organo-antimony compounds are now being used in the treatment of Kala-azar and other diseases, and both the qualitative tests and quantitative determination are important from the point of view of drug standardization. Antimony is also used as a glaze in enamel ware and it may be dissolved by acid food or drink and cause poisoning. Its detection in food or stomach contents is, therefore, frequently required. For its detection the Reinsch test can be usefully carried out and the characteristic deposit of needle-shaped crystals, often found in star-shaped clusters of Sb_2O_3 , distinguishes Sb from As (see figs. 44 and 62 on pages 424 and 572).

Lead.—The detection and estimation of very small quantities of Pb in presence of organic matter in cases of lead poisoning are of great significance in cases connected with the administration of Workmen's Compensation Act and in medico-legal work. The method of detection is similar in details to the method of estimation and it is, therefore, described later (see pp. 576-586).

Iron.—The organic matter is destroyed by heating the substance in a crucible when a residue consisting of metallic iron and its oxides is obtained. It is dissolved by warming with HCl containing a little HNO_3 and filtered. The filtered solution which contains the iron in the ferric state is tested either by KCNS or by potassium ferro-cyanide as described before.

DETERMINATION OF THE ELEMENTS

Determination of Carbon and Hydrogen.—It depends upon the *principle* that if a known weight of the substance is completely oxidized by heating in a current of air or oxygen in presence of heated cupric oxide the C and H are oxidized quantitatively to carbon dioxide and water. These are absorbed by a strong solution of caustic potash and anhydrous calcium chloride respectively and the percentages of C and H are calculated from the weights of CO_2 and H_2O obtained.

The air and oxygen are kept in two reservoirs which are connected with two separate rows (only one of which is shown in Fig. 10) of wash bottles and drying tubes placed side by side so that either the air or oxygen can be used for the combustion. The oxygen (or air) is freed from moisture by first passing through a wash-bottle containing concentrated sulphuric acid and then through a U-tube containing a long layer of granulated soda-lime and a short layer of calcium chloride. It then bubbles through a small wash-bottle containing concentrated sulphuric acid, which helps to find out the rate at which the gas is passing through the combustion tube.

The combustion tube (Fig. 9) consists of a hard glass tube about 12 mm. inside diameter and of such length (commonly about 90-95 cm.) that it projects about 5 cm. on either side of the combustion furnace in which it is placed. On the side where the oxygen or air enters there is placed a long roll of oxidized copper gauze, then a space for putting a porcelain or platinum *boat* holding the substance, then a long column of copper oxide (in wire form) held in position by small asbestos plugs and finally another roll of oxidized copper gauze.

The combustion tube is connected at the other end with a weighed U-tube containing granular anhydrous calcium



FIG. 9.

chloride and a weighed potash bulb containing a concentrated solution (about 50 per cent) of KOH. A small calcium

chloride tube is attached to the potash bulb in order to prevent the escape of any water vapour from the potash solution.

The combustion furnace (Fig. 10) consists of an iron framework having a row of Bunsen burners, the flame of each of which can be regulated independently, and there is an iron trough above the burners over which is placed the glass combustion tube.

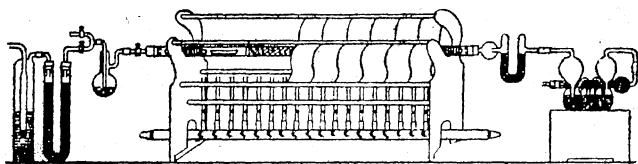


FIG. 10.

Before finally connecting the CaCl_2 tube and KOH -bulb, the combustion tube is heated in a current of air to free it from all organic matter and then cooled. About 0.2 gram of the substance is weighed accurately in a porcelain or platinum boat and placed in the combustion tube. All the parts are then carefully connected as described above with rubber corks and tubes and a slow current of air is allowed to pass. The burners below the long column of CuO are first lighted and this part made nearly red hot. The substance in the boat is then carefully heated from one side and the combustion allowed to proceed slowly, first in a current of air and then in a current of oxygen. The H_2O formed is absorbed in the weighed CaCl_2 tube and the CO_2 evolved is absorbed in the weighed KOH bulb. When the combustion is over and all the moisture and carbon dioxide driven into the absorption vessels, the furnace is allowed to cool in a current of air. The absorption vessels are then taken out and carefully weighed. The increase in weight gives the weight of H_2O and CO_2 from which one can easily calculate the percentage of H and C .

Some Modifications in the Determination of C and H.
If the substance contains *nitrogen* the roll of oxidized copper gauze in the front end of the combustion tube is replaced

by a longer copper gauze which is reduced to metallic copper before each combustion and kept hot in order to reduce any oxide of nitrogen to gaseous nitrogen and thus prevent its absorption by the solution of potash.

If the substance contains a *halogen*, a spiral of silver gauze is used instead of the copper gauze in order to decompose any volatile copper halide which may be produced and also to retain any free halogen as silver halide which is less volatile.

If the substance contains *sulphur*, the layer of copper oxide is replaced by a layer of granular, fused lead chromate mixed with about one-fourth of its bulk of wire-form copper oxide. The oxides of sulphur are decomposed by the lead chromate and retained as lead sulphate.

If the substance is a *liquid* it is taken in a small tube with a loose stopper or if the liquid is very volatile it is weighed in a sealed glass bulb with a capillary end which is broken before introduction into the combustion tube.

Determination of Oxygen.—There is no direct method of determining the percentage of oxygen. It is obtained by subtracting the sum of the percentages of the other elements from 100.

Example.—0.2004 gram of glucose gave on combustion 0.2940 g. of CO_2 and 0.1202 g. of H_2O ; find the percentage composition. Since 44 g. of CO_2 would be obtained from 12 g. of C, 0.2940 g. of CO_2 would be obtained from

$0.2940 \times \frac{12}{44}$ or 0.0802 g. of C. Hence the percentage of

C = $\frac{0.0802 \times 100}{0.2004} = 40.01$. Again, since 18 g. of H_2O would be obtained from 2 g. of H, 0.1202 g. of H_2O would be obtained from $0.1202 \times \frac{2}{18}$ or 0.0133 g. of H. Hence the

percentage of H = $\frac{0.0133 \times 100}{0.2004} = 6.63$. The percentage

of O is, therefore, by difference = $100 - (40.01 + 6.63) = 53.36$.

Determination of Nitrogen

I. Dumas' Method.—This method is applicable to all organic compounds and depends upon the *principle* that the compound is oxidized in a combustion tube with cupric oxide in a current of pure carbon dioxide. The nitrogen evolved is collected in a nitrometer over a concentrated solution of caustic potash which absorbs all the carbon dioxide, and the percentage of nitrogen is calculated from the volume of nitrogen gas found.

About 0.2 gram of the dry substance is weighed accurately, intimately mixed with an excess of finely powdered pure dry cupric oxide and introduced into the combustion tube in the space where the porcelain boat is placed. The other arrangements inside the combustion tube are practically the same as in the determination of C and H, only the oxidized copper gauze at the front end is replaced by a long copper gauze which is reduced to metallic copper before each combustion in order to decompose the oxides of nitrogen to free nitrogen. Instead of air or oxygen, a current of pure CO_2 , free from air, from a Kipp's apparatus, is passed and the nitrogen gas evolved is collected in a *Schiff's nitrometer* (Fig. II) containing a concentrated solution (about 50 per cent) of KOH. The potash solution absorbs all the carbon dioxide and the volume of nitrogen is read. The observed volume is reduced to N.T.P. and the percentage of N is calculated from this.

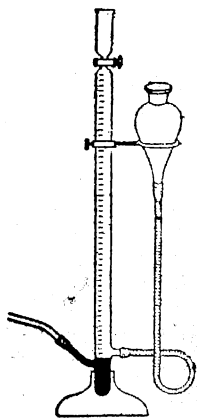


FIG. II.

Example. Compound with C, H, N & O; 0.1805 gram of acetanilide gave on combustion 0.4706 g. of CO_2 and 0.1083 g. of H_2O ; and 0.2 gram of the substance gave 18.06 c.c. of N_2 at 22°C and 750 mm. pressure; calculate the percentage composition. Weight of C = $\frac{0.4706 \times 12}{44} = 0.1283$;

hence $\text{C} = \frac{0.1283 \times 100}{0.1805} = 71.08$ per cent by weight of

$H = 0.1083 \times \frac{2}{18} = 0.01203$; hence $H = \frac{0.01203 \times 100}{0.1805}$
 $= 6.66$ per cent. The volume of N at N.T.P. =
 $18.06 \times \frac{750}{760} \times \frac{273}{273 + 22} = 16.5$ c.c. Now, since the gram-
 molecular weight of any gas occupies 22240 c.c. at
 N.T.P., 28 grams of nitrogen gas (N_2) would occupy the
 same volume. Hence the weight of 16.5 c.c. of N_2 ,
 $= \frac{28 \times 16.5}{22240} = 0.02077$ and the percentage of N
 $= \frac{0.02077 \times 100}{0.2} = 10.38$ per cent. The percentage of oxygen
 found by difference = $100 - (71.08 + 6.66 + 10.38) = 11.88$
 per cent.

2. Kjeldahl's Method.—The method depends upon
 the fact that when an organic compound is heated with con-
 centrated sulphuric acid the nitrogen present is converted
 into ammonium sulphate. The ammonia is then liberated
 from this by an excess of caustic soda and is determined by
 absorbing it in a known volume of a standard sulphuric
 acid.

The method is very simple in its operation, involves
 inexpensive apparatus and allows a number of determina-
 tions to be carried out simultaneously. It is widely used in
 the estimation of proteins in foodstuffs, analysis of fertili-
 zers, therapeutic compounds, etc., and is thus of value in
 biochemical, agricultural and other analyses. Especial
micro-apparatus has also been devised for working with very
 small quantities of material in biochemical analysis.

This method does not, however, yield such accurate
 results as may be necessary for the determination of
 formulæ, and it fails in the case of organic compounds like
 nitro, nitroso-, azo- or diazo-compounds, hydrazines,
 osazones, nitrate esters, cyanides, etc.

A small amount (0.5 gram or more, depending upon
 the content of N) of the dry substance, weighed accurately,
 is taken in a long necked, round bottom flask, known
 as Kjeldahl flask, with about 20 c.c. of concentrated
 pure sulphuric acid, about 10 grams of pure anhydrous
 potassium bisulphate $KHSO_4$ (which raises the boiling point

of the H_2SO_4 and hastens the oxidation) and a small crystal of copper sulphate (or KMnO_4 , metallic Hg, selenium oxide, etc.), which acts as a catalyst, and heated until the liquid becomes almost colourless. The flask is provided with a loose glass stopper and the heating is carried out inside a fume cupboard. The clear solution is cooled, diluted with about 200 c.c. of ammonia-free water and transferred completely to a round bottom flask connected to a vertical condenser through a bulb-trap which prevents the splashing of the alkaline solution into the condenser (Fig. 12). A little zinc dust is added to prevent bumping and the end of the condenser tube dips just below the surface of the standard acid with 2 drops of a suitable indicator kept in a conical flask. A strong solution of NaOH (about 40 per cent) is carefully added until the solution is alkaline as indicated by a piece of litmus paper already introduced into the flask and the solution is then distilled until all the ammonia is absorbed by the acid. The residual acid is ascertained by back titration with a standard alkali. A *blank* experiment is always recommended and the titration result is deducted from that of the actual experiment.

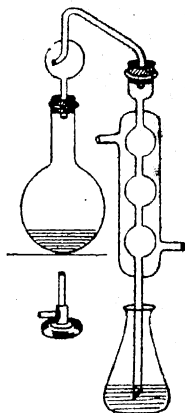


FIG. 12.

Example. The ammonia evolved from 0.50 gram of wheat flour was absorbed in 25 c.c. of $\text{N}/10 \text{H}_2\text{SO}_4$; the acid required 18.36 c.c. of $\text{N}/10 \text{NaOH}$ for back titration; calculate the percentage of nitrogen and of the protein present.

The ammonia evolved is equivalent to $(25.00 - 18.36)$ or 6.64 c.c. of $\text{N}/10 \text{H}_2\text{SO}_4$, which is equivalent to 6.64 c.c. of $\text{N}/10$ ammonia solution. But 1 c.c. of $\text{N}/10$ ammonia solution contains 0.0014 gram of N. Hence the amount of N in 0.50 g of the sample $= 0.0014 \times 6.64 = 0.009296$ g. and the percentage of N $= \frac{0.009296 \times 100}{0.50} = 1.85$ per cent.

Now proteins contain about 16 per cent of N; hence the percentage of N obtained by the Kjeldahl method, when

multiplied by the factor $\frac{100}{16}$ or 6.25, gives the percentage of protein. The percentage of protein in the present sample is, therefore, $1.85 \times 6.25 = 11.56$ per cent.

Determination of Halogens, Carius' Method.

The method depends upon the *principle* that when an organic compound is heated at a high temperature in a sealed tube with fuming nitric acid in presence of silver nitrate, the organic portion is oxidized and the halogen is converted into a silver halide, which can be easily separated and weighed.

A thick walled soft glass tube, about 18 inches long and about half an inch in internal diameter is sealed at one end.

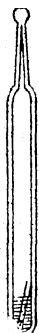


FIG. 13.

About 5.0 c.c. of pure fuming nitric acid are carefully introduced into the bottom of the tube and about 0.5 gram of pure crystals of silver nitrate dropped in. About 0.2 gram of the substance is weighed accurately in a narrow glass tube about 3 inches long and sealed at one end, and the tube is allowed to slide slowly down the large tube so that the acid does not come in contact with the substance. The open end of the large tube is then carefully melted and drawn out to a capillary end with a thick wall and sealed (Fig. 13). When cool, the glass tube is put inside an iron tube and heated in a *bomb furnace* (Fig. 14) at about 250° for several hours. When

cool and while still inside the iron tube within the furnace, the projecting tip of the capillary end is warmed cautiously with a Bunsen flame till the glass softens and the pressure of the gases inside is released by a small opening. The tube is then taken out of the iron case and the capillary end broken. The silver halide formed is transferred to a weighed filter paper, washed carefully until free from AgNO_3 and HNO_3 , dried and weighed.

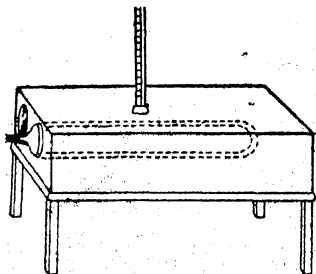


FIG. 14.

Example. 0.15 gram of iodoform gave 0.2682 gram of AgI; calculate the percentage of iodine; Ag=107.88.

$$\text{Weight of iodine} = \frac{126.92 \times 0.2682}{234.8} \text{ g.}$$

$$\therefore \text{percentage of iodine} = \frac{126.92 \times 0.2682 \times 100}{234.8 \times 0.15} = 96.64 ;$$

calculated for CHI_3 = 96.72 per cent.

Determination of Sulphur

(a) **Carius' Method.**—The substance is heated as before in a sealed tube in the bomb furnace with pure fuming HNO_3 but without any AgNO_3 . The sulphur present is converted into sulphuric acid and it is precipitated as BaSO_4 by the addition of BaCl_2 . The BaSO_4 is collected on a weighed filter paper, washed, dried and weighed, the percentage of S being calculated from the weight of BaSO_4 .

(b) **Fusion Method.**—About 0.2 gram of the substance is fused in a crucible with a mixture of anhydrous sodium carbonate and sodium peroxide. The melted mass is cooled, extracted with water acidified with HCl and treated with a solution of BaCl_2 . The BaSO_4 formed is weighed as before.

Example. 0.25 gram of thiourea gave 0.7670 g of BaSO_4 ; calculate the percentage of S; Ba=137.4; S=32.

$$\text{Weight of sulphur} = \frac{32 \times 0.7670}{233.4} \text{ g.}$$

$$\therefore \text{percentage of sulphur} = \frac{32 \times 0.7670 \times 100}{233.4 \times 0.25} = 42.06 ;$$

calculated for CSN_2H_4 = 42.10 per cent.

Determination of Phosphorus.—The substance is heated in a sealed tube with pure fuming nitric acid in a bomb furnace as in Carius' method. The phosphoric acid formed is precipitated by magnesia mixture (an aqueous solution of NH_4Cl , NH_4OH & MgCl_2) as magnesium ammonium phosphate $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ which is transferred to a filter, washed and dried. The precipitate is heated in a crucible and the percentage of P is calculated from the amount of magnesium pyrophosphate $\text{Mg}_2\text{P}_2\text{O}_7$ formed.

Determination of Arsenic.—See pp. 555—561, Chap. 36.

Determination of Lead.—See pp. 585—586, Chap. 36.

Percentage Composition, Empirical formula, and Molecular formula.

We have seen before that the percentage composition of an organic compound can be calculated from a quantitative analysis. By dividing the percentage of each element by its atomic weight, we can get the *relative numbers of atoms* in the molecule.

Taking, for *example*, the figures from p. 16, C=40.01 per cent, H=6.63 per cent, and O=53.36 per cent, the relative number of C atoms = $40.01 \div 12 = 3.3$, the relative number of H atoms = $6.63 \div 1 = 6.63$, and the relative number of O atoms = $53.36 \div 16 = 3.3$. Now since fractions of atoms cannot exist, we can get the *relative whole numbers* by dividing each figure by the lowest amongst them. Thus, C = $3.3 \div 3.3 = 1$, H = $6.6 \div 3.3 = 2$, and O = $3.3 \div 3.3 = 1$. Hence the simplest formula is CH_2O .

Again, taking the *example* on p. 17, C=71.08 per cent, H=6.66 per cent, N=10.38 per cent, and O=11.88 per cent, the relative numbers of atoms are: C = $71.08 \div 12 = 5.92$, H = $6.66 \div 1 = 6.66$, N = $10.38 \div 14 = 0.74$, and O = $11.88 \div 16 = 0.74$. Dividing each of these by the lowest figure we get, C = $5.92 \div 0.74 = 8$, H = $6.66 \div 0.74 = 9$, N = $0.74 \div 0.74 = 1$ and O = $0.74 \div 0.74 = 1$. Hence the simplest formula is $\text{C}_8\text{H}_9\text{NO}$.

The simplest formula obtained from the percentage composition is known as the *empirical formula* (Gk. *em-peira*-trial). It only gives the relative numbers of the different elements present in the molecule. The formula giving the *actual numbers* of atoms in the molecule, the *molecular formula*, may be the same or any multiple of the empirical formula since the percentage composition would be the same for all. If, for instance, the molecular weight of the first compound is found to be 180, the molecular formula would be $\text{C}_6\text{H}_{12}\text{O}_6$ (see Chapter 3).

CHAPTER III

DETERMINATION OF MOLECULAR WEIGHT

It has been stated earlier that the *empirical formula* indicates the *relative numbers* of the different kinds of atoms present in the molecule, whereas the *molecular formula* expresses the *actual numbers* of these atoms, and that the molecular formula is found out from the empirical formula by determining the molecular weight of the substance. For example, it was shown that the empirical formula for glucose as determined by analysis was CH_2O , and that the molecular formula must be $(\text{CH}_2\text{O})_n$ where n may be any whole number. A determination of the molecular weight gave the value 180 and hence the molecular formula must be $(\text{CH}_2\text{O})_6$, i.e., $\text{C}_6\text{H}_{12}\text{O}_6$, the molecular weights corresponding to the values 1, 2, 3, 4, 5 and 6 for n being 30, 60, 90, 120, 150 and 180 respectively. The determination of the *exact* molecular weight is not, however, essential and it suffices if the method enables one to decide about the correct multiple of the empirical formula. And in the determination of the empirical formula it may be remembered as a general rule that the "*total number of valencies* in a molecule must be an *even number*." Thus a molecular formula like $\text{C}_{18}\text{H}_{35}\text{O}$ is incorrect since the total number of valencies is 101.

A. PHYSICAL METHODS

1. From Vapour Density.

Victor Meyer Method.—The method consists in vaporizing a weighed quantity of the substance in an enclosed space and measuring the volume of air it displaces. The vapour density is calculated from this, the molecular weight being twice the vapour density compared to hydrogen as unity. The method is applicable only to compounds which can be volatilized without decomposition at the atmospheric pressure.

The apparatus consists of an inner tube *ab* with a narrow side tube *c*, the mouth of the inner tube being fitted with a rubber cork. A graduated glass tube *d* filled with water is inverted over the end of the side tube in a trough filled with water (Fig. 15). The inner tube is

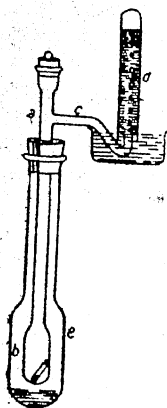


FIG. 15.

surrounded by an outer jacket *e* which holds a liquid (e.g., water, aniline, nitrobenzene, etc.) whose boiling point is about 40° higher than that of the substance whose molecular weight is to be determined. The cork fixing the inner tube to the outer jacket has a vertical groove to allow for the expansion of air inside the jacket. About 0.1 gram of the substance is weighed accurately in a small stoppered bottle known as *Hofmann's bottle*. The liquid in the jacket is boiled until the evolution of air bubbles from the end of the side tube *c* has ceased. The graduated tube *d* filled with water is then placed

over the side tube and the loosely stoppered bottle containing the substance is dropped into the inner tube by quickly opening the rubber stopper and again inserting it tightly. The substance vaporizes and the vapour displaces the air which bubbles out and collects in the graduated tube. When the evolution of air bubbles has ceased, the volume of air is measured.

Example. 0.05 gram of ethyl alcohol displaced 27.22 c.c. of air at 25°C and 755 mm. pressure; calculate the molecular weight; vapour tension of aqueous vapour at 25°C is 23.55 mm. Volume of air at

$$\text{N.T.P.} = 27.22 \times \frac{755 - 23.55}{760} \times \frac{273}{273 + 25} = 24.0 \text{ c.c.}$$

Hence 24.0 c.c. of alcohol vapour weigh 0.05 gram. Now 1 c.c. of hydrogen weighs 0.00009 gram; hence 24.0 c.c. of hydrogen weigh $0.00009 \times 24 = 0.00216$ g; so the vapour density of the substance compared to hydrogen as unity =

$\frac{0.05}{0.00216} = 23.1$, and the molecular weight = 46.2 . The molecular weight calculated from the formula $\text{C}_2\text{H}_6\text{O} = 46.0$.

2. From Depression of the Freezing Point.

The Cryoscopic Method.—The method depends upon the fact that when a substance is dissolved in a liquid with which it does not react, it will depress the freezing point of the liquid by an amount depending upon the molecular weight of the dissolved substance. The depression caused by dissolving a *gram molecule* (i.e., molecular weight expressed in grams) of a substance in 100 grams of the solvent is found to be a constant and is known as the *molecular depression* of the solvent. Thus the values for molecular depression are: water 18.6°, benzene 5.0°, acetic acid 39.0°, etc., and the relation between the molecular weight and the depression of the freezing point is given by the equation, $M = K \times \frac{W}{D}$, where M = molecular

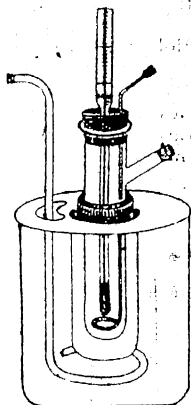


FIG. 16.

weight, W = weight of substance dissolved in 100 grams of solvent, and D = depression observed. The apparatus (Fig. 16) consists of a stout glass jar provided with a large stirrer. A wide test tube passes through the central hole of a metallic cover for the jar and serves as an air-jacket. A narrower tube with a side tube passes through the wide test tube and is held in position by a cork. The narrow tube is fitted with a special thermometer, known as *Beckmann thermometer*, and a small stirrer. The Beckmann thermometer has a range of about 6 degrees, each degree being divided into hundredths. There is a small reservoir on the top (Fig. 17) which allows the zero position of the mercury column to be adjusted to any convenient position of the thermometer scale. About 15 to 20 grams of the solvent are weighed carefully and introduced into the inner test tube.



FIG. 17.

Its freezing point is determined by cooling it with a freezing mixture (usually ice and salt) placed in the jar. The solvent is then slightly warmed up by the hand and the

weighed substance (about 0.3 gram) introduced by the side tube and dissolved. The freezing point of the solution is determined again and the difference gives the depression of the freezing point.

Example. 0.2371 gram of an alkaloid dissolved in 14.5 grams of benzene lowered the freezing point of benzene by 0.230° ; calculate the molecular weight. Molecular depression of benzene $= 50^{\circ}$.

$$\text{Mol. wt.} = 50 \times \frac{0.2371 \times 100}{14.5 \times 0.230} = 355.5; \text{calculated M.W.} = 356.0.$$

N. B. The cryoscopic method gives abnormal results in the case of acids, salts, etc., which *dissociate* in the solvent or in the case of substances which *associate* or form molecular aggregates in the solvent used.

3. From Depression of Melting Point.

Rast's Camphor Method. A Micro Method. The method is based upon the fact that the molecular depression for camphor is very high (400°) compared to any of the solvents used in the determination of molecular weight by the cryoscopic method. It consists in the determination of the melting point of a sample of pure camphor (m.p. about 176°C) in which a known weight of the substance has been dissolved. The method enables one to carry out the determination of the molecular weight with very small amounts of the material, i.e., as a micro method, with a fair degree of accuracy. It is rapid and can be carried out without the use of any special apparatus and with an ordinary thermometer.

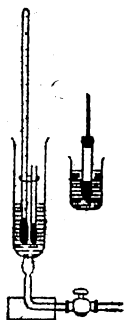


FIG. 18.

A few milligrams of the organic substance are mixed with about 10 times its bulk of camphor, both being weighed accurately. The mixture is melted rapidly in a small closed tube (Fig. 18) to a clear homogeneous solution by dipping into a bath of concentrated sulphuric acid heated to about 175°C . The mixture is allowed to cool, powdered, taken in a capillary tube and the melting point is determined in a small apparatus using a small flame (Fig. 18), the temperature being raised very slowly. The temperature at which the last trace of crystalline material just disappears is taken as the melting point. The melting point of the sample of camphor used is also determined in a similar manner and the difference in the melting point gives the depression.

Example. 6.4 milligrams of acetanilide dissolved in 61.0 milligrams of camphor showed the melting point 144.5° ; the m.p. of the sample of camphor was 176°C ; calculate the molecular weight.

$$D = 176.0 - 144.5 = 31.5; K = 400; W, \text{ the weight of the substance in 100 g. of camphor} = \frac{6.4 \times 100}{61.0} = 10.491 \text{ grams. Hence,}$$

$$\text{M.W.} = \frac{400 \times 10 \cdot 491}{31 \cdot 5} = 133 \cdot 2 ; \text{calculated for } \text{C}_6\text{H}_5\text{NO} = 135.$$

4. From Elevation of Boiling Point.

The Ebullioscopic Method.—This method depends upon the fact that when a substance is dissolved in a liquid it will raise the boiling point of the liquid by an amount depending upon the molecular weight of the dissolved substance. The *molecular elevation*, *i.e.*, the rise in boiling point when a gram molecular weight of the substance is dissolved in 100 grams of the solvent, is for water $5 \cdot 2^\circ$, chloroform $36 \cdot 6^\circ$, benzene $26 \cdot 1^\circ$, and so on, and the molecular weight is obtained from the equation $M = K \times \frac{W}{E}$, where M is the molecular weight, K is the molecular elevation, W is the weight of the substance dissolved in 100 grams of the solvent and E is the elevation observed.

B. Chemical Methods.

1. By Titration

(a) **Acids.**—If the basicity of the acid is known the molecular weight can be determined from the amount of standard alkali required to neutralize a known weight of the acid. For example, if the acid is monobasic, the weight which is neutralized by 40 grams of NaOH (its gram molecular weight) gives the molecular weight of the acid.

(b) **Bases.**—If the acidity of the base is known, the molecular weight can be determined from the amount of standard acid required to neutralize a known weight of the base. The molecular weights of strong organic bases like alkaloids are sometimes determined in this way.

2. **By Silver Salts.**—The method is applicable to organic *acids* which yield sparingly soluble silver salts and whose *basicity* is known. The silver salt is prepared by adding a solution of silver nitrate to a solution of the ammonium salt. It is then carefully purified and dried. A known weight of the dry silver salt is next gradually heated to redness in a porcelain crucible by which the organic matter is burnt off and pure metallic silver is left behind which is weighed.

Example. 0.30 gram of the silver salt of a monobasic acid gave 0.1643 gram of Ag; calculate the molecular weight; $\text{Ag} = 107.88$.

$$\text{The percentage of silver} = \frac{0.1643 \times 100}{0.30} = 54.77 ; \text{hence the}$$

percentage of the organic component = $100 - 54.77 = 45.23$, and the amount combined with one gram atom of silver = $45.23 \times \frac{107.88}{54.77} = 89.1$. Now since the acid is monobasic one atom of hydrogen must have been replaced by one atom of Ag, and therefore the molecular weight of the acid = $89.1 + 1 = 90.1$.

3. **By Platinum Salts.**—The method is applicable to organic bases, such as amines, alkaloids, etc., which give sparingly soluble double salts with platinic chloride and whose acidity is known. These salts, known as platinichlorides or chloroplatinates, have the general formula $(B.HCl)_2PtCl_4$ where B is a monacid base, and $B_2HCl.PtCl_4$ where B is a diacid one.

The platinic chloride of the base is prepared by treating a soluble salt of the base with a solution of platinic chloride. The precipitated salt is carefully washed and dried. A known weight of the dry salt is then carefully ignited in a porcelain crucible and the residue of pure platinum weighed.

Example. 0.2493 gram of the platinic chloride of a diacid base gave 0.0635 g. of Pt as residue; calculate the molecular weight; $Pt = 195.2$. The mol. wt. of the platinic chloride, $B_2HCl.PtCl_4$, = $\frac{0.2493 \times 195.2}{0.0635} = 766.3$. Subtracting the weight of H_2PtCl_6 (409.9). the molecular weight of the base B = $766.3 - 409.9 = 356.4$.

CHAPTER IV

STRUCTURE OF ORGANIC COMPOUNDS

The formation of innumerable organic compounds containing only a limited number of elements depends, as has been mentioned earlier, upon the *tetravalency* of the carbon atom and the power of the carbon atom to *combine with each other* to form long chains or rings.

The four valencies of the carbon atom are distributed symmetrically in space and that is best visualized by assuming (see Chapt. 15) that the carbon atom occupies the *centre* of a *regular tetrahedron* while the four valencies are directed towards its four *corners* (Fig. 19). For simplicity, however, the valencies are represented by short lines or dots irrespective of the directions of the valencies in space. Thus the simplest compound of C and H, methane CH_4 , is represented by

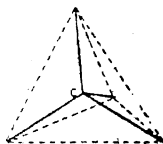
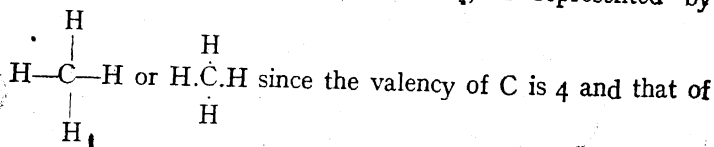
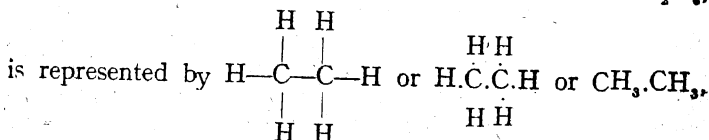


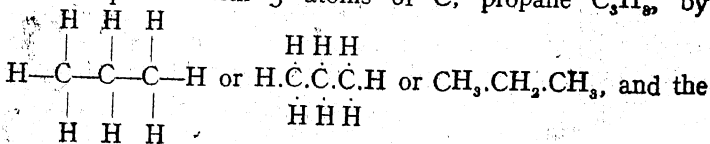
FIG. 19.



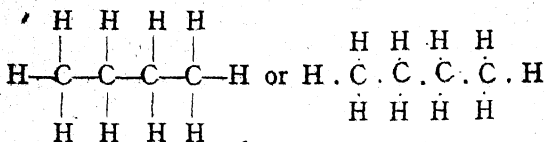
H is 1. The compound with 2 atoms of C, ethane C_2H_6 ,



the compound with 3 atoms of C, propane C_3H_8 , by



compound with 4 atoms of C, butane C_4H_{10} , by



or $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_3$, and so on.

The elaborate formulæ showing the individual valencies by lines or dots are known as *graphic formulæ* or *constitutional formulæ*.

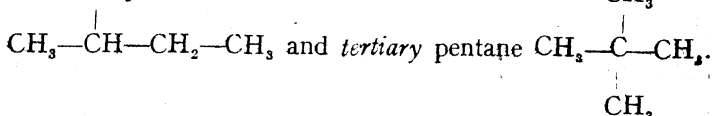
We have seen that we can get a long series of compounds of C and H with an open chain. These compounds of C and H, known as *hydrocarbons*, are called *saturated hydrocarbons* since the valencies of the C atoms are all saturated or satisfied. We observe another interesting relationship between the above hydrocarbons. We see that each compound differs from the previous member by a constant group of atoms, *viz.*, CH_2 , and we expect this difference from the valency of C itself. They can be represented by the general formula $\text{C}_n\text{H}_{2n+2}$ and the physical properties of the members show a regular gradation. Thus the boiling point or density is found to rise gradually with increase in the number of C atoms and so on. A series of compounds like this, belonging to the same chemical group, differing from each other by the same group of atoms and showing a regular gradation of physical properties, is known as a *homologous series* (Gk. *homos*—same, *logos*—relation) and the individual members are known as *homologues*.

Similar homologous series are met with amongst many other classes of carbon compounds such as alcohols, aldehydes, acids, esters, etc. The similarity of chemical properties and the gradation of physical properties in these homologous series introduce more system in organic chemistry and make its study easier.

When we deal with higher hydrocarbons we observe another interesting phenomenon. Thus butane C_4H_{10} , the compound with 4 atoms of C, can be arranged in two different

ways $\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{CH}_3$ and $\text{CH}_3-\overset{\text{CH}_3}{\underset{|}{\text{CH}}}-\text{CH}_3$. The

former with an unbranched chain is known as *normal* butane, and the latter with a branched chain as *isobutane*. These compounds, which have the same percentage composition and the same molecular weight but different physical properties and structural formulæ, are known as *isomers* (Gk. *isos*—equal, *meros*—part). When we come to the next higher hydrocarbon, pentane C_5H_{12} , we get three isomers, *normal* pentane $CH_3-CH_2-CH_2-CH_2-CH_3$, *isopentane*



The number of isomers goes on increasing with the number of C atoms, and we theoretically expect 5 hexanes, 9 heptanes, and so on.

In certain special cases, two isomers may be readily convertible into each other, usually due to the presence of a mobile hydrogen atom in the molecule. This kind of isomerism is known as **tautomerism** (*tautos*—same, *meros*—part) and the two forms are known as *tautomers*. Since the two forms usually exist together in a state of dynamic equilibrium, they are also known as *dynamic isomers* and the isomerism spoken of as *dynamic isomerism*. Thus ethyl-acetoacetate is known to possess the *keto* form $CH_3.CO.CH_2.COOC_2H_5$ and the *enol* form $CH_3.C(OH):CH.COOC_2H_5$ (see Chapt. 15). Again hydrocyanic acid is found to exist in the isomeric forms $H.C:N$ and $H.N:C$, the former giving rise to *nitriles* and the latter to *isonitriles* (see Chapt. 19).

We have seen that the structural formula for methane is CH_4 . When treated with iodine it gives methyl iodide CH_3I , and if CH_3I is treated with an alkali hydroxide it gives methyl alcohol CH_3OH . Again two molecules of CH_3I when treated with metallic sodium combine with each other to give ethane $CH_3.CH_3$. Similarly, ethane C_2H_6 gives rise to the series of compounds C_2H_5I , C_2H_5OH , $C_2H_5.C_2H_5$, and so on. We thus observe that groups of atoms like CH_3 , C_2H_5 , etc., behave like single atoms and pass unchanged from one compound to another. These

atomic groups are known as **radicals** or *radicles* (*radix*—root). The monovalent hydrocarbon *radicals* like methyl CH_3 —, ethyl C_2H_5 —, propyl C_3H_7 —, butyl C_4H_9 —, amyl C_5H_{11} —, etc., are known as *alkyl radicals* or alkyl groups. We will come across various types of radicals as we proceed with the study of organic chemistry, and we shall find that the properties of a organic compound depend upon the properties of the various radicals present in the molecule. Some of the common radicals met with in organic chemistry are as follows, the short lines indicating their valencies: —OH (hydroxyl); —COOH (carboxyl); —CHO (aldehyde); —NH₂ (amino); =NH (imino), =CO (carbonyl), —CO.CH₃ (acetyl); —NO₂ (nitro); —CN (cyanogen), etc. Some of the radicals found amongst the group of compounds known as aromatic compounds are as follows: —C₆H₅— (phenyl); C₆H₅.CH₂— (benzyl); C₆H₅.CO— (benzoyl); C₆H₅.CH= (benzal); —N=N— (azo), etc.

ELECTRONIC THEORY OF VALENCY

Atoms and Valency Electrons.—According to the electronic theory an atom is an electrically neutral system and consists of some units of positive charges known as *protons* and an equal number of units of negative charges known as *electrons*. The protons are confined to the nucleus of the atom and the electrons are distributed on the surface of imaginary spheres or shells surrounding the nucleus. The nucleus of an atom also contains a certain number of neutral bodies or *neutrons* which vary not only in individual elements but in the individual atoms of the same element, for example, the nucleus of hydrogen atoms may contain 0 to 2 neutrons and the nucleus of chlorine atoms 17 to 20 neutrons and so on. The variation in the number of neutrons in the nucleus of atoms of a particular element is responsible for the phenomenon of *isotopy*. It is therefore evident that a nucleus containing only protons and neutrons always possesses a certain amount of positive charge which is neutralised by the same amount of negative charge of the electrons situated outside the nucleus.

The mass of an atom depends upon the number of protons and neutrons contained in the nucleus since a proton is about 1950 times heavier than an electron and a neutron has got a mass equal to that of a proton. The amount of positive charge or in other words the number of protons in the nucleus or the number of electrons in the shells is known as the *atomic number* of the element.

As the number of protons or electrons increases with the increasing atomic number of the elements, more and more of these shells or layers of electrons are formed round the nucleus. In hydrogen there is one shell containing only one electron and there is only one proton in the nucleus. In helium there is also one shell but it contains two electrons. In lithium there are 3 electrons, but they are not situated in one shell, two of them forming the first shell and the third one forming the second shell. It is stated that the first shell never contains more than two electrons which form what is called a 'duplet.' In the second shell the number of electrons varies from one to the maximum of eight (or 'octet' as it is termed). In the third shell the number may go up to 18 and in the fourth it may be as many as 32.

In incomplete shells, *i.e.*, the shells in which the number of electrons is less than eight, the electrons are held in a less stable condition than those forming part of a complete shell or octet. These unstable electrons are more mobile and free to move to some other atom to bring about a chemical union of the atoms concerned. It is these mobile electrons which determine the valency of the atom and are, therefore, known as *valency electrons*. The table on p. 34 gives an idea of the distribution of electrons in the atoms of some common elements.

It will be seen in the following table that the shells of helium, neon and argon are complete and they possess no mobile or valency electrons and as such they are inert, *i.e.*, incapable of entering into chemical combination with other elements. Lithium, sodium and potassium on the other hand possess one each, carbon contains four, nitrogen five, oxygen six and chlorine seven valency electrons.

When an atom gives up an electron, it becomes an ion charged with one unit of positive electricity (electro-positive ion) whereas

Elements.	Mass = Number of protons & neutrons in the nucleus of the diff. isotopes.	Atomic number = Number of protons in the nucleus	Number of electrons in the atom.	Distribution of electrons in the atom				
				1st shell	2nd shell	3rd shell	4th shell	5th, 6th & 7th shells.
Hydrogen	1, 2, 3	1	1	1
Helium	4	2	2	2	0
Lithium	7, 6	3	3	2	1
Beryllium	9, 8	4	4	2	2
Boron	11, 10	5	5	2	3
Carbon	12, 13	6	6	2	4
Nitrogen	14, 15	7	7	2	5
Oxygen	16, 18, 17	8	8	2	6
Fluorine	19	9	9	2	7
Neon	20, 21, 22, 23	10	10	2	8	0
Sodium	23	11	11	2	8	1
Magnesium	24, 25, 26	12	12	2	8	2
Phosphorus	31	15	15	2	8	5
Sulphur	32, 33, 34	16	16	2	8	6
Chlorine	35, 37	17	17	2	8	7
Argon	40, 36	18	18	2	8	8	0	...
Potassium	39, 41	19	19	2	8	8	1	...
Iron	56, 54	26	26	2	8	14	2	...
Arsenic	75	33	33	2	8	18	5	...
Platinum	195	78	78	2	8	18	32	16, 2
Mercury	202, 200 & seven others	80	80	2	8	18	32	18, 2
Lead	208, 206, 207 & five others	82	82	2	8	18	32	18, 4
Uranium	238, 235 & seven others	92	92	2	8	18	32	18, 12, 2

when the atom gains an electron, it becomes an ion charged with one unit of negative electricity (electro-negative ion). When there are only a few electrons in the incomplete or outer shell, as for example, in H, Li, Mg, K, etc., (having one or two electrons), the atom tends to give up its electrons to other atoms while in the case of elements with a nearly complete outer shell (*e.g.*, oxygen with 6, or chlorine with 7 electrons in the outer shell), the atom takes up electrons from other atoms. It may, therefore, be stated that the electro-positive character of an element depends upon its capacity to lose electrons and the electro-negative character on its power to gain electrons. Thus Na, Li, etc., with one valency electron which is readily given up by the atom, are strongly electro-positive and behave as monovalent elements whereas F, Cl, etc., having seven valency electrons in each and thus having accommodation for one additional electron to complete the outer shell, have a strong tendency to take up an electron and are, therefore, strongly electro-negative monovalent elements.

Similarly calcium having 2 valency electrons to spare

is electro-positive or oxygen with accommodation for two additional electrons is electro-negative and both are divalent elements.

If an element gives up its valency electrons in entering into chemical union with another element, it is termed to possess positive valency and if an element takes up electrons from another element and becomes electro-negative, it is distinguished as possessing negative valency. Thus the negative valency of F or Cl is one, *i.e.*, its atom with seven valency electrons tends to complete the octet by taking up only one electron, but since it has seven electrons which it might give up, its positive valency will be seven. Similarly, the positive valency of Li or Na is one and as it can accommodate seven electrons to complete the octet, it will have a negative valency of seven and so on. In these extreme cases, the tendency in one direction is very much stronger than in the other direction and that is why compounds of lithium showing heptavalent negative character or of fluorine showing heptavalent positive character are not known.

In cases of elements not occupying extreme positions as stated above, we find chemical compounds in which the elements exhibit both these positive and negative characters. For example, carbon with four valency electrons is capable of combining with four atoms of hydrogen to form the compound CH_4 in which carbon behaves as a tetravalent negative element, while in the case of CCl_4 it exhibits its tetravalent positive character. In accordance with this theory, every element, therefore, possesses two valencies, a positive valency and a negative valency, and the sum of these is equal to eight.

Chemical combination between two elements.—It has been stated before that chemical combination between two atoms takes place through the agency of valency electrons. An atom of chlorine having space for only one extra electron in its outer shell, is believed to link up with an atom such as sodium, which has only one electron to give. Similarly, an atom of oxygen with six valency electrons in its outer shell can link up with two atoms, each of which has only one valency electron, *viz.*, H_2O or Na_2O . It is, therefore,

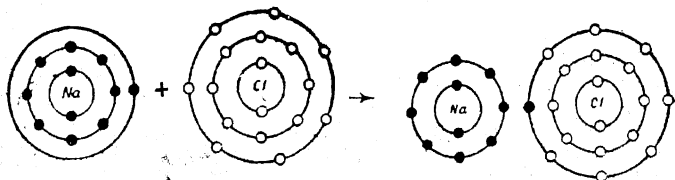
evident that atoms tend to link up with other atoms in such a way that they may be surrounded with stable or complete shells—an octet or a duplet as the case may be.

There are three main *types of inter-atomic linkage or union* :—

- (1) Electro-valent or ionizable linkage or polar bond.
- (2) Covalent or non-ionizable linkage or non-polar bond.
- (3) Co-ordinate linkage, or semipolar bond.

(1) **Electro-valent linkage.**—This type of union is brought about by transference of electrons from one atom to another and is characteristic of compounds which are electrolytes, *i.e.*, compounds which ionize on solution. As an example of this kind of union, the combination of sodium and chlorine may be mentioned. Here the atom of sodium transfers its only valency electron from its outer shell (3rd shell) to the chlorine atom having 7 valency electrons. In this transaction the sodium atom becomes a positively charged sodium ion, with its 3rd shell disappearing and leaving the 2nd shell (an octet) to indicate its stable structure. On the other hand, the chlorine atom after gaining an electron from the sodium atom becomes a negatively charged chlorine ion with its unstable outer shell converted into a stable octet. The two ions of Na and Cl are held together by electrostatic forces which weaken in solution and the ions separate.

The above reactions are represented by the following diagrams—



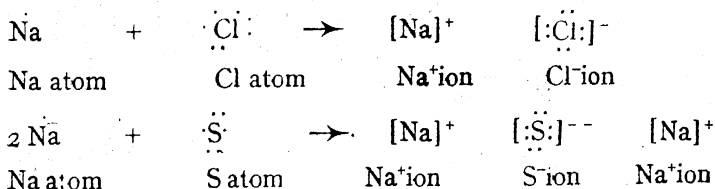
Na atom.
2nd shell—an
octet.
3rd shell in-
complete,
having one
electron.

Cl atom.
2nd shell—
an octet,
3rd shell in-
complete,
having 7
electrons.

Na⁺ ion.
2nd shell—
an octet.
3rd shell dis-
appears.

Cl⁻ ion.
3rd shell
becomes
complete—
an octet.

It is customary to represent the linkage by showing the valency electrons only in the following manner—

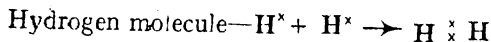


Carbon does not ordinarily give up or take up electrons in the above manner and does not, therefore, give rise to ionizable compounds. In organic chemistry this type of linkage is confined to acids, bases and salts, that is, those which ionize on solution.

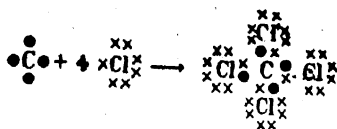
(2) **Covalent linkage.**—In this type there is no transference of any electron from one atom to the other, but the union is formed by the sharing of pairs of electrons between the two atoms concerned each of which provides one electron to the pair and each pair contributes towards the stability of both the combining atoms by bringing them to the level of the nearest inert element having the stable shell of a duplet or an octet. The products of this type of union are non-ionizable molecules and are most common amongst organic compounds. The simplest example of such a union is the formation of the hydrogen molecule in which the two hydrogen atoms share a pair of electrons between them and form a stable shell of two electrons, a duplet (helium type), around the hydrogen atoms.

Methane, CH_4 , for instance, is formed by the sharing of a pair of electrons by each hydrogen atom with the carbon atom thus completing the stable shell of eight electrons, an octet of neon type (vide table, p. 34) around the carbon atom and stable shells of two electrons (helium type) around the hydrogen atoms. Similarly, CCl_4 is formed by the sharing of a pair of electrons by each chlorine atom with the carbon atom and stable shells of octets are thus formed around each of the combining atoms. The above unions are represented below diagrammatically by showing only the outer or valency electrons and indicating their source by a cross (x), a dot

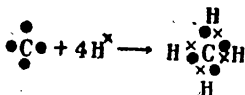
(·) or a circle (°), e.g., × for hydrogen, fluorine, chlorine and methyl, · for carbon and nitrogen and ° for oxygen and boron electrons. It may, however, be noted that the electrons are not static and do not occupy particular positions in the atom as shown in the diagrams but they are believed to be in a state of rapid motion. The shared electrons have been shown by placing them between two contributing atoms:—



Carbon tetrachloride, CCl_4

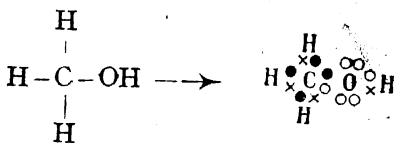


Methane, CH_4

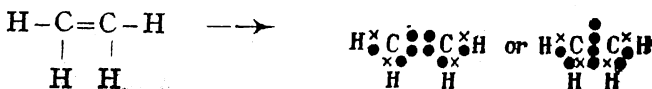


Electronic formulæ of some other organic compounds.

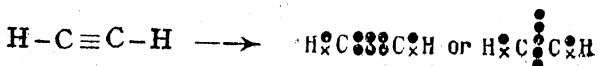
Methyl alcohol—



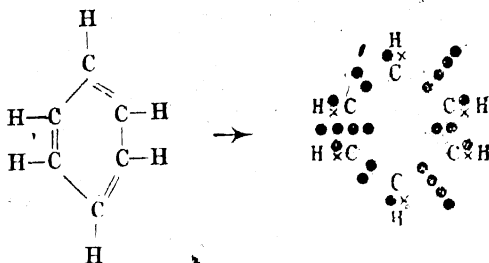
Ethylene—



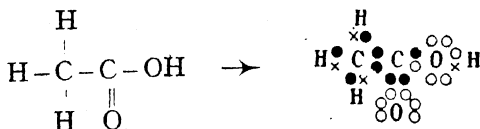
Acetylene—



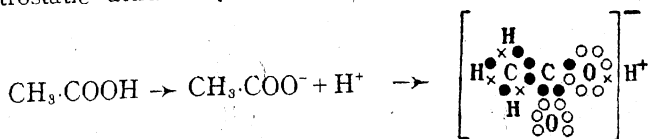
Benzene—



Acetic Acid—

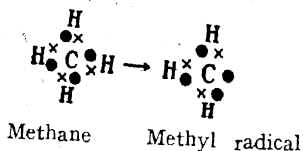


As acetic acid is an electrolyte and ionizes on solution into CH_3COO^- and H^+ ions, it may also be represented in the following manner in which electrovalency also plays an important role, the union of carbon atoms with H and O in the CH_3COO^- ion being effected by the method of covalent linkage (non-polar bond) as described above, while the H^+ ion being formed by electrovalent linkage or polar bond. Here the electron of the hydrogen atom of COOH is transferred to the oxygen atom to produce the stable octet around the latter, and hydrogen being thus deprived of its negative charge becomes a positively charged ion and is held in combination with the negative ion CH_3COO^- by electrostatic attraction.



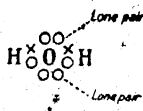
An alkyl radical possesses one unshared valency electron, and is therefore equivalent to a hydrogen atom. Methyl

radical, for example, may be shown in the following way—

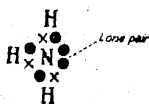


(3) **Co-ordinate linkage.**—This type of union is formed by the sharing of a pair of electrons, but, unlike the covalent linkage, both electrons are contributed by one of the atoms. The atom which contributes the electrons (the donor) contains at least one pair of electrons which are not attached to any other atom of the molecule and are called 'lone pair'. The other atom (the acceptor) takes up the 'lone pair' to form the stable structure or octet. The examples of 'lone pair' of electrons may be found in oxygen of water or in nitrogen of ammonia. In the former, there are two pairs which help in the union of water with certain salts in the form of water of crystallization, and in the latter there is one pair which also helps ammonia in forming complex compounds with other molecules, e.g., union of NH_3 with BF_3 . In BF_3 , which is a stable compound, boron has not got a completed octet, and so takes up the two electrons provided by the N atom in NH_3 . The donor on account of having failed to receive its own share of the electrons from its partner, as happens in the covalent linkage, becomes electropositive and the acceptor becomes electro-negative. This kind of linkage is depicted by a short arrow and the direction of the arrow indicates that both the electrons are provided by the donor. A typical example of this type of linkage in organic compounds is the formation of trimethylamine oxide. Originally, this type was termed 'semi-polar double bond' since it appears to possess the combined properties of covalence and electrovalence.

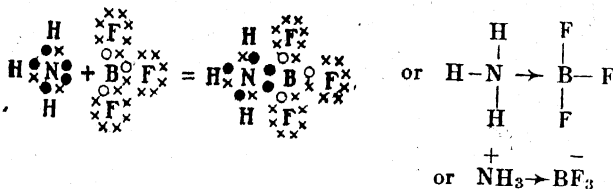
(i) Water



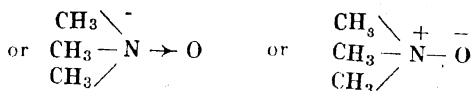
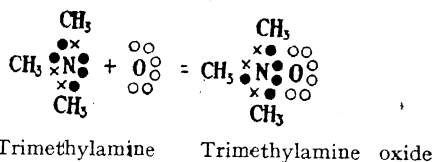
(ii) Ammonia



(iii)

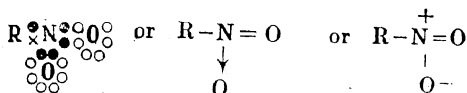


(iv)



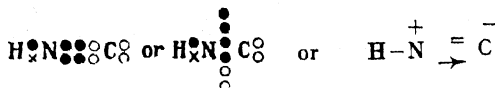
Nitro compounds, isocyanides, etc., are also important examples of co-ordinate linkage:—

Nitro Compounds, *e.g.*, $\text{R} \cdot \text{NO}_2$



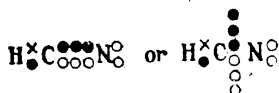
In this molecule, one of the oxygen atoms and the nitrogen atom share two pairs of electrons which are contributed equally by both the atoms but the other oxygen atom and the nitrogen share one pair of electrons which are contributed entirely by the nitrogen atom, which, therefore, behaves as a donor towards the second oxygen atom but not towards the first, and as such the arrow indicates the donor, the electropositive character of which is depicted by the + sign and the electronegative character of the acceptor is indicated by - sign.

iso-Hydrocyanic acid:—



In this molecule, nitrogen and carbon share 3 pairs of electrons of which two pairs are contributed by nitrogen and only one pair by carbon. The nitrogen atom, therefore, acquires electropositive character while the carbon atom becomes electronegative.

In the case of hydrocyanic acid, C and N also share 3 pairs of electrons, but they contribute half and half and none of them, therefore, behaves as a donor or an acceptor. Thus,



From what has been discussed in the foregoing pages, it is evident that the ordinary structural formulæ of organic compounds may be converted into electronic formulæ by replacing one single non-ionizing bond by a pair of shared electrons, each ionizing bond by the transfer of an electron, and each double bond by four shared electrons (except in cases of co-ordinate linkage).

It may, however, be stated that the octet rule does not apply to elements beyond the first three periods, and in some cases not even to these elements. For example, the formation of SF_6 can only be explained by assuming that S is surrounded by 12 electrons ($:\ddot{\text{S}} + 6\ddot{\text{F}}:$). Similarly, in the compounds BF_3 , BCl_3 , etc., B is surrounded by 6 electrons ($:\ddot{\text{B}} + 3\ddot{\text{Cl}}:$) and not by a complete octet. They are stable compounds and thus form exceptions to the rule.

PART II

ALIPHATIC COMPOUNDS

CHAPTER V

SATURATED HYDROCARBONS

The saturated hydrocarbons described in this chapter are known as *hydrocarbons of the methane series*. The name "methane series" is derived from the fact that the first member of this series is called 'methane'.

They are also known as *paraffin hydrocarbons* or simply *paraffins* (*parum*-little, *affinis*-affinity), since they are very stable and inert substances and are not easily acted upon by common chemical reagents. They form the following *homologous series* with the general formula $C_n H_{2n+2}$ and are designated with the suffix 'ane'; thus:

CH_4	Methane	$C_{10}H_{22}$	Decane	$C_{30}H_{62}$	triacontane
C_2H_6	Ethane		etc., etc.,	$C_{31}H_{64}$	hentriacontane
C_3H_8	Propane	$C_{15}H_{32}$	Pentadecane		etc., etc.,
C_4H_{10}	Butane	$C_{16}H_{34}$	Hexadecane	$C_{35}H_{72}$	Pentatriacontane
C_5H_{12}	Pentane		etc., etc.,		etc., etc.,
C_6H_{14}	Hexane	$C_{20}H_{42}$	Eicosane	$C_{60}H_{122}$	Hexacontane
C_7H_{16}	Heptane	$C_{21}H_{44}$	Heneicosane		etc., etc.,
C_8H_{18}	Octane		etc., etc.,	$C_{\infty}H_{190}$	Is the heaviest
C_9H_{20}	Nonane	$C_{25}H_{52}$	Pentacosane		paraffin known
			etc., etc.,		

Starting from methane (b.p. $-164^{\circ}C$), the lowest member of the series, they are gases at the ordinary temperature up to 4 C atoms (butane, b.p. $+1^{\circ}C$). The members from 5 C atoms (pentane, b.p. $+36^{\circ}C$) to 16 C atoms (hexadecane, m.p. $+18^{\circ}C$) are liquids, while members above 16 C atoms are solids.

Occurrence and Natural Formation.—The lower members, especially methane, occur in marshy places, in coal seams and in gases from petroleum wells, being known as

natural gas. Various higher members are found in crude petroleum.

Two theories are prevalent as to the origin of petroleum in nature: (1) According to Engler it is produced by the decomposition of the fossil remains of animal and vegetable matters at very high temperatures and pressures within the interior of the earth, and (2) according to Mendeléeff it is produced by the action of steam upon metallic carbides formed in the deeper strata of the earth. The fact that most of the specimens of petroleum contain small amounts of organic compounds having nitrogen and sulphur and that they show a slight optical activity seem to support the former theory.

Petroleum, Rock Oil, Mineral Oil. Petroleum (Gk. *petra*-rock, *oleum*-oil) occurs in Pennsylvania (U.S.A.), Mexico, Venezuela (South America), Canada, Caspian Sea district (Southern Russia), Galicia, Roumania, Iraq, Iran, Japan, Burma and Borneo. In India it is found in Digboi (Assam), Sylhet (E. Bengal) and in Khaur near Rawalpindi (W. Punjab). More than 60 per cent of the world production is from U.S.A., about 10 per cent from Venezuela, about 10 per cent from Russia, and about 5 per cent from Iran and Iraq. The oil-bearing stratum is tapped by deep boring, sometimes to a depth of more than 10,000 ft. There is first of all an escape of gases containing the lower hydrocarbons like methane, ethane, etc., followed by crude petroleum which comes out as a brownish looking, viscid, fluorescent liquid with a disagreeable smell. This is generally carried by long iron pipes to some convenient place, generally a sea-coast, where the crude oil is refined before export.

Petroleum consists principally of saturated hydrocarbons but the composition of the oil varies greatly with the natural source. Thus the oil from Pennsylvania consists mostly of hydrocarbons of the *methane series* $C_n H_{2n+2}$ whereas Russian petroleum is found to be rich in hydrocarbons of the *naphthene series* $C_n H_{2n}$, which are cyclic polymethylenes such as cyclopentane, cyclohexane, etc. Petroleum also contains small amounts of *unsaturated hydrocarbons* such as olefines and acetylenes or *aromatic hydrocarbons* as in

Borneo oil and smaller amounts of organic compounds containing *nitrogen and sulphur*.

The crude oil is first separated into three principal fractions by distillation in large iron stills: (1) *benzine* or *petroleum naphtha*, b.p. up to 150°C , (2) *kerosene* or *illuminating oil*, b.p. 150° — 300°C , and (3) *residuum* in the still, b.p. above 300°C . These fractions are purified by *chemical treatment* with *concentrated sulphuric acid* to remove unsaturated hydrocarbons and nitrogenous bases, with *caustic alkalis* to remove acid bodies and then with a solution of *litharge* in NaOH to remove sulphur compounds and finally *distilled* fractionally.

The different fractions of benzine or petroleum naphtha are given different trade names. In the American oil the first fraction, technically known as *cymogene* and *rhigolene* (b.p. up to 30°C ; sp. gr. 0.64), which consists mainly of butane and pentane, is used for refrigeration and also for local anæsthesia by freezing. The second fraction (b.p. 30° — 70°C ; sp. gr. 0.65) known as *petroleum ether* or *light petroleum* consisting mainly of pentane and hexane, is used as a solvent for fats and oils. A fraction of petroleum ether suitable for use in India and other tropical countries can be made by redistilling the commercial petroleum ether and collecting the fraction between 40° — 60°C . The third fraction (b.p. 70° — 120°C ; sp. gr. 0.72, known as *petrol*, *ligroin* or *gasoline*, consisting mainly of hexane, heptane and octane, is used as *motor spirit*, the fuel for motor cars and aeroplanes, and also for the dry cleaning of clothes. The next fraction (b.p. 120° — 150°C ; sp. g. 0.76), known as *petroleum benzine* or *benzoline*, consisting mainly of octane and nonane, is used as a solvent and for dry cleaning. (N.B. The *benzine* from petroleum, which consists of a mixture of open chain hydrocarbons, should not be confused with the pure aromatic hydrocarbon *benzene* having a ring structure and obtained mainly from coal tar).

The second fraction obtained from crude petroleum yields *kerosene* or *illuminating oil*. It is carefully purified by washing with concentrated sulphuric acid, dilute caustic soda, etc., and then distilled, the fraction boiling between

150°–300°C being collected. The American oil consists of a mixture of open chain saturated hydrocarbons containing 10 to 18 carbon atoms, and is used as an illuminant in lamps, as fuel, as larvicide and as a solvent or diluent for insecticides like pyrethrum, D.D.T., etc.

Kerosene used for illuminating purposes should not contain any volatile fractions which may form an explosive mixture with the air inside the oilpot of a lamp causing accidents. The standard for these oils is fixed by the *flash-point* which is defined as the minimum temperature at which a sample of petroleum yields inflammable vapour which will produce a momentary flash when tested with a standard flame in a special apparatus known as the Abel's apparatus. In the *Abel's Flash-point Apparatus*, the oil is kept in a small covered metal cup which is slowly heated up in a water jacket. There is a slit in the cover of the cup which is opened from time to time by an automatic device and a small flame is also automatically introduced near the slit. When the flash-point is reached the vapour ignites with a small explosion which puts out the flame and the temperature of the oil is noted. The flash-point adopted for Great Britain is 73°F (22.8°C). In warmer climates, the flash-points should naturally be higher. In India the flash point taken is 76°F for petrol and other dangerous petroleum and 95°F for kerosene.

The residue in the still after the distillation of crude petroleum up to 300° still contains valuable products such as *liquid paraffin*, lubricating oils, vaseline, paraffin wax, etc. This is distilled in vacuum and the various products are decolorized by passing through charcoal filters. The B.P. *liquid paraffin* is a colourless, odourless and tasteless liquid consisting of a mixture of hydrocarbons (sp. gr. 0.880 to 0.895 at 15.5°/15.5°C) and is used in medicine as a mild laxative. The fraction above 300°, known as *lubricating oil*, is used as oil fuel in Diesel engines, as batching oil in jute mills and as lubricants for machinery. *Vaseline* or *petroleum jelly* (m.p. 35°–45°C) is found either as a colourless or a yellowish semi-solid mass, and is used as a lubricant and in the preparation of various medicinal ointments. *Paraffin wax* is used in candle-making and in histological work for preparing sections of tissues. In B.P., it is known in two

forms, *soft paraffin*, m.p. 40° – 46°C , and *hard paraffin* m.p. 50° – 60°C . Paraffin wax, which is a mixture of saturated hydrocarbons having a high molecular weight, should not be confused with genuine *waxes*, such as bees-wax, which are esters of higher alcohols and higher fatty acids (see p. 171). *Ozokerite* is a solid paraffin resembling paraffin wax in composition and properties and occurs naturally in Galicia, its purified quality being known as *ceresine*.

Shale Oil.—A kind of soft bituminous clayey rock or shale, occurring in Scotland, New South Wales, Tasmania and other places, is found to yield when distilled large quantities of oil containing products similar to those derived from petroleum together with inflammable gases, ammonia liquor and tar. These shales are probably the residues of fossil fish and other marine organisms.

Cracking of Petroleum.—This is a process by which high boiling fractions of petroleum are converted into low boiling products of lower molecular weight. The vapour is led through special chambers at a high temperature and pressure in presence of a catalyst. The so-called 'fluid catalyst' generally refers to a powdered aluminium silicate which is carried along with the vaporized hydrocarbons through the reaction chamber. It is recovered, reactivated, and returned to the process.

By the cracking of *kerosene* in thick-walled red hot iron retorts, gaseous saturated and unsaturated hydrocarbons are produced which are employed as a *substitute for coal gas* for use in laboratories in smaller towns where there is no gas supply.

The cracking of petroleum not only helps to increase the yield of the proper quality of motor fuel, known as *gasoline*, petrol, or motor spirit, but also yields various products of economic value by varying the temperature, pressure and catalyst. One may thus obtain *ethylene* $\text{CH}_2:\text{CH}_2$, used in preparing ethyl alcohol, acetic acid, ethyl chloride, lead tetraethyl, etc.; *propene* $\text{CH}_3.\text{CH}:\text{CH}_2$, used in preparing isopropyl alcohol, acetone, glycerol, etc.; *butene* $\text{CH}_3.\text{CH}_2.\text{CH}:\text{CH}_2$, used to prepare butadiene from which

Buna type of synthetic rubber is made ; *iso-octane* used in motor fuel; etc. ; *isobutylene*, which is the starting material for butyl rubber ; *aromatic hydrocarbons*, such as toluene $C_6H_5.CH_3$, which is the source of drugs, dyes and explosives; and so on.

Octane Number.—In the preparation of motor fuel, care is taken that the petrol does not cause sudden and violent detonation, known as *knocking*, in internal combustion engines, especially when it is to be used for aviation. Normal heptane $CH_3.(CH_2)_6.CH_3$ knocks very badly and is stated to have an octane number of 0, while *iso-octane* (2 : 2 : 4-trimethyl pentane) $CH_3.CH(CH_3).CH_2.C(CH_3)_2.CH_3$, which does not cause any knocking, is stated to possess an octane number of 100. The gasoline prepared by the cracking of petroleum is tested for its anti-knocking properties and the fuel used for aviation purposes must have an octane number near about 100.

The addition of small amounts of *lead tetraethyl* $Pb(C_2H_5)_4$, a *very toxic* compound prepared by the action of ethyl chloride upon an alloy of lead and sodium, reduces the knocking properties of gasoline. Small quantities of its solution in ethylene dibromide are therefore added to motor fuels and gasoline mixed with lead tetraethyl is coloured with a dyestuff (for aviation, blue ; for others, red) in order to serve as a warning for its high toxicity.

Nomenclature of Saturated Hydrocarbons.—As mentioned in a previous chapter, those hydrocarbons in which the carbon atoms form a straight chain are designated with the prefix *normal* (*n*-) and those with branched chain with the prefix *iso*-. According to the international system of nomenclature, the *normal* paraffins retain their former names and in the case of branched chains the longest normal chain decides the name, and the branches are taken as substituents.

Thus, $\overset{8}{CH_3}.\overset{7}{CH_2}.\overset{6}{CH_2}.\overset{5}{CH_2}.\overset{4}{CH_2}.\overset{3}{CH_2}.\overset{2}{CH_2}.\overset{1}{CH_3}$ would be called *n-octane*.

$CH_3.CH_2.CH_2.CH_2.CH_2.CH_2.CH_2.CH_3$, 2-methyl heptane,

CH_3 ,

$CH_3.CH_2.CH_2.CH_2.CH_2.CH_3$, 3-ethyl hexane,

C_2H_5

CH_3 ,

CH_3 ,

$CH_3.CH.CH_2.\overset{\cdot}{C}.CH_3$, 2 : 2 : 4-trimethyl pentane,

CH_3 .

or *iso-octane*, and so on.

Physical and Chemical Properties of Saturated Hydrocarbons.—As described before, the lower members (methane, ethane, propane and butane) are gases, the intermediate ones (pentane, hexane, etc.), are liquids and the higher members (heptadecane, etc.) are solids. The lower members have a faint odour while the higher ones are almost odourless. They are almost insoluble in water but soluble in ether. Absolute alcohol dissolves the lower members easily but the solubility decreases in the case of solid members with gradual increase of molecular weight. They are all lighter than water, the specific gravity varying from about 0.4 to about 0.8.

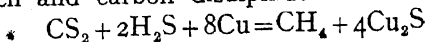
The gases form explosive mixtures with air and the liquids are inflammable. They are very inert towards chemical reagents. Concentrated sulphuric acid, caustic potash, potassium permanganate and even fuming nitric acid and chromic acid have no action in the cold. Chlorine and bromine act upon them, especially in the presence of diffused sunlight, giving substitution products. They burn in the presence of air or oxygen with the formation of carbon dioxide and water.

Methane, Marsh Gas, CH_4 .

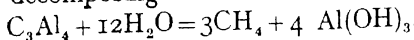
Occurrence.—This gas is formed in nature by the bacterial decomposition of organic matter under water and issues from marshy places, hence called *marsh gas*. The *natural gas* from petroleum wells consists mainly of methane. It is found in coal mines as *pit-gas* (*fire-damp*) where it causes disastrous accidents due to its forming an explosive mixture with air. About 6 per cent of methane is necessary to produce explosion. The presence of coal dust in air accelerates the action and even 3 per cent of methane is enough for explosion in the presence of coal dust. A higher percentage of methane produces a flash only. The coal-gas formed by the destructive distillation of coal may contain about 35 to 40 per cent of methane. It is also found in the human intestinal gases which may contain more than 55 per cent of methane.

Synthesis and Preparation—

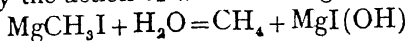
(1) By passing a mixture of the vapours of sulphuretted hydrogen and carbon disulphide over red-hot copper:



(2) By decomposing aluminium carbide with water:

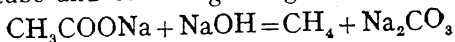


(3) By the action of water on magnesium methyl iodide:



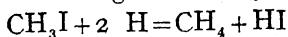
N.B.—Magnesium alkyl halides of the type MgCH_3I are known as *Grignard's reagents*. They have the general formula R.MgX , where R is an alkyl radical and X a halogen atom. They are formed by the action of a dry alkyl halide upon magnesium ribbons suspended in *dry* ether. These reagents are very reactive and serve as the starting materials for the synthesis of various types of organic compounds.

(4) *In the laboratory*, it can be easily prepared by heating an intimate mixture of one part of anhydrous sodium acetate with about four parts of dry soda-lime in a hard glass test tube and collecting the gas over water:



The methane thus produced is not quite pure and may contain appreciable amounts of hydrogen and ethylene. The reaction of heating the Na-salt of an acid is a general one and can be used for the preparation of higher members of this series, *e.g.*, Na-propionate would yield ethane, and so on.

(5) *Pure methane* can be prepared in the laboratory by reducing methyl iodide with zinc-copper couple and dilute alcohol, the latter liberating nascent hydrogen:



This is a general reaction and can be used for the preparation of higher hydrocarbons.

The *zinc-copper couple* is prepared by treating pure granulated zinc with a dilute solution of copper sulphate. The zinc, which is covered with a fine deposit of copper, is washed with water and then with alcohol. The zinc-

copper couple and some alcohol containing a drop or two of dilute sulphuric acid are placed in a flask fitted with a dropping funnel containing methyl iodide and with a cylinder funnel containing some zinc-copper couple moistened with alcohol. The methyl iodide is added drop by drop and the methane produced is collected over water (Fig. 20). Any vapour of methyl iodide which escapes is decomposed by the zinc-copper couple in the cylinder funnel.

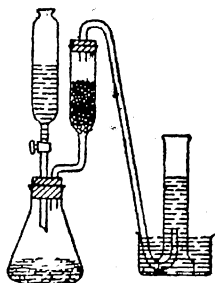
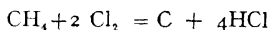
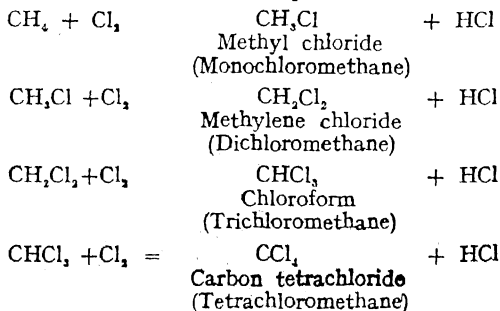


FIG. 20.

Properties and Reactions.—It is a light, colourless and odourless gas. It burns with a faintly luminous flame and forms an explosive mixture with certain proportions of air or oxygen. It is very slightly soluble in water (1 litre at 4° dissolves 49 cc. of the gas) but is moderately soluble in absolute alcohol. When cooled by liquid air it forms a colourless liquid, b. p.—164°. Methane is a very stable and inert gas and is not affected by reagents like concentrated nitric acid, fuming sulphuric acid, bromine water, caustic potash, or potassium permanganate. In the dark, chlorine has no action on methane but in *direct sunlight*, the following reaction takes place with an explosion:



In *diffused sunlight*, there is no explosion and the chlorine atoms gradually displace the hydrogen atoms of methane forming different *substitution products*:



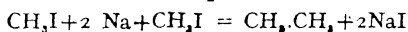
With bromine, but not with iodine, a similar series of reactions take place forming methyl bromide CH_3Br , methylene bromide CH_2Br_2 , bromoform CHBr_3 and carbon tetrabromide CBr_4 .

Ethane, Dimethyl, C_2H_6 , $\text{CH}_3\cdot\text{CH}_3$.

Occurrence.—In natural gas obtained from petroleum wells it may occur to the extent of about 10 per cent. It is also found in very small amounts in coal gas.

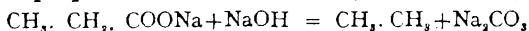
Synthesis and Preparation.—

(1) It can be synthesized by heating methyl iodide with sodium dissolved in dry ether:

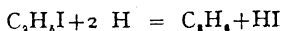


This general reaction, known as *Wurtz Reaction*, shows the constitution of methane and can be used for the preparation of higher hydrocarbons.

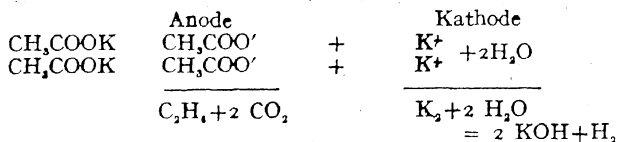
(2) It can be prepared in the laboratory by heating sodium propionate with soda-lime (*vide* methane):



(3) In the pure state it can be prepared in the laboratory by reducing ethyl iodide with zinc-copper couple exactly in the same manner as in the case of methane:



(4) By the electrolysis of potassium acetate or aqueous acetic acid under suitable conditions:



This reaction shows that a fatty acid with n carbon atoms gives a hydrocarbon with $2n-2$ carbon atoms.

Properties and Reactions.—It is a light, colourless and odourless gas and burns with a faintly luminous flame. With air or oxygen it forms an explosive mixture. Liquefied ethane boils at -89° . Ethane is practically insoluble in

water but is fairly soluble in absolute alcohol. Like methane it is not affected by most reagents. In diffuse sunlight chlorine acts upon ethane giving *substitution products* such as ethyl chloride $\text{CH}_3\text{CH}_2\text{Cl}$, ethylene dichloride $\text{CH}_2\text{Cl}\cdot\text{CH}_2\text{Cl}$, acetylene tetrachloride $\text{CHCl}_2\cdot\text{CHCl}_2$, etc.

Some Higher Saturated Hydrocarbons

Propane, C_3H_8 , $\text{CH}_3\text{CH}_2\text{CH}_3$: occurs in crude petroleum; colourless gas; b.p. -45° ; sp. gr. 0.515 at 16° ; 100 vols. H_2O dissolve 6.5 vols. at 17.8° and 753 mm.

n-Butane, C_4H_{10} , $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$: occurs in crude petroleum; colourless gas; b.p. $+1^\circ$; 1 vol. H_2O dissolves 0.15 vol. at 17° and 772 mm.

Isobutane, Trimethyl methane, C_4H_{10} , $\text{CH}(\text{CH}_3)_3$: occurs in Canadian and Roumanian petroleum; colourless gas; b.p. -11.5° ; 1 vol. H_2O dissolves 0.13 vol. at 17° and 772 mm.

n-Pentane, C_5H_{12} , $\text{CH}_3(\text{CH}_2)_3\text{CH}_3$: occurs in American petroleum; colourless liquid; b.p. 36° ; sp. gr. 0.6337 at 15° .

Isopentane, Dimethyl ethyl methane, C_5H_{12} , $(\text{CH}_3)_2\text{CH}\cdot\text{CH}_2\cdot\text{CH}_3$: occurs in American petroleum; colourless liquid; b.p. 30° ; sp. gr. 0.6251 at 14.4° .

Tetramethyl methane, C_5H_{12} , $\text{C}(\text{CH}_3)_4$: occurs in Caucasian and Roumanian naphtha; colourless liquid; b.p. 9.5° .

n-Hexane, C_6H_{14} , $\text{CH}_3(\text{CH}_2)_4\text{CH}_3$: occurs in American petroleum; colourless liquid; b.p. 69° ; sp. gr. 0.6606 at 20° .
etc., etc.,

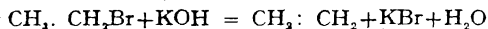
n-Octane, C_8H_{18} , $\text{CH}_3(\text{CH}_2)_6\text{CH}_3$: occurs in petroleum; colourless liquid; b.p. 126° ; sp. gr. 0.7036 at 20° .
etc., etc.

Pentacosane, $\text{C}_{25}\text{H}_{52}$: occurs in hard paraffin obtained from brown coal; colourless solid, m.p. 54° .

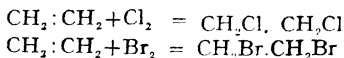
CHAPTER VI

UNSATURATED HYDROCARBONS

We have seen that the formula for ethane, a saturated hydrocarbon, is $\text{CH}_3 \cdot \text{CH}_3$, and that it yields ethyl bromide $\text{CH}_3 \cdot \text{CH}_2\text{Br}$ by the substitution of one atom of hydrogen by bromine. Now, when ethyl bromide is heated with an *alcoholic solution* of KOH it acts as follows and yields a hydrocarbon with 2 hydrogen atoms less than in ethane:

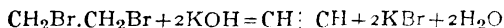


Unlike ethane, which gives substitution products, the hydrocarbon ethylene $\text{CH}_2 : \text{CH}_2$, thus formed reacts readily with chlorine or bromine and gives *additive compounds* such as ethylene dichloride $\text{CH}_2\text{Cl} \cdot \text{CH}_2\text{Cl}$ or ethylene dibromide $\text{CH}_2\text{Br} \cdot \text{CH}_2\text{Br}$:

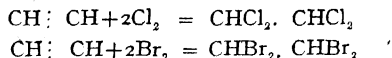


This property is due to the fact that the valencies of the two carbon atoms are not satisfied, or in other words, ethylene is an *unsaturated compound*.

Again, if we heat ethylene dibromide with a solution of alcoholic potash, the following reaction takes place and another hydrocarbon with 2 atoms of hydrogen less than in ethylene is formed:



This hydrocarbon, acetylene $\text{CH} : \text{CH}$, reacts like ethylene readily with chlorine or bromine giving additive compounds and is thus an unsaturated hydrocarbon:



The *unsaturated* hydrocarbons contain less hydrogen atoms than the corresponding *saturated* hydrocarbons with the same number of carbon atoms, and they give *additive*

products instead of *substitution products*. The state of unsaturation is represented by *double bond* or *triple-bond* between the carbon atoms, the triple bond showing a higher degree of unsaturation than the double bond as is clearly shown by the action of chlorine upon acetylene and ethylene. The graphic representation by double and triple bond between carbon atoms should not, therefore, be mistaken as indicating added strength but rather as a *state of strain* accompanied by *instability* and greater *reactivity* of the carbon atoms.

This state of strain or tension has been well expressed by the *strain theory of Baeyer*. The four valency bonds of a carbon atom may be assumed to be directed towards the four corners of a regular tetrahedron, the carbon atom being situated in the centre (Fig. 21).

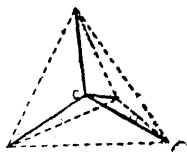


FIG. 21

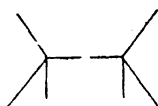


FIG. 22(a)



FIG. 22(b)



FIG. 22(c)

The valency directions are thus equally distributed in space and the angle between two such directions can be calculated to be $109^{\circ} 28'$. When the carbon atoms are singly linked there is no bending of the valency directions since the carbon atoms can rotate freely (Fig. 22a). When, however, there is a double bond, the valency directions suffer distortion and are thus subjected to a state of strain (Fig. 22b). This tension is proportional to the degree of distortion and will naturally increase with a triple bond (Fig. 22c). As a result of this state of strain these unsaturated compounds are more unstable and tend to pass into the saturated state, and, therefore, show greater chemical reactivity.

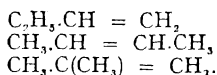
The *presence of unsaturation* in a compound can be demonstrated practically by treating it with (1) a dilute (about 0.1 per cent) aqueous solution of *potassium permanganate* made slightly alkaline with sodium carbonate, the permanganate solution being decolorized, or (2) *bromine*

water, which is readily decolorized. If the substance is insoluble in water, it can be dissolved in carbon tetrachloride and treated with a dilute solution of bromine in carbon tetrachloride.

There are two important series of unsaturated hydrocarbons: (1) the *ethylene series*, with a double bond, forming the following *homologous series*:

Ethylene or ethene $\text{CH}_2=\text{CH}_2$, propylene or propene $\text{CH}_3.\text{CH}=\text{CH}_2$, n-butylene or butene $\text{CH}_3.\text{CH}_2.\text{CH}=\text{CH}_2$, n-amylene or pentene $\text{CH}_3.\text{CH}_2.\text{CH}_2.\text{CH}=\text{CH}_2$, etc.

Theoretically, methylene CH_2 forms the first member of this series but it has no free existence. Its polymeric ring compounds, known as polymethylenes or cycloparaffins, do exist. Ethylene is also known as *olefiant gas* (oil-forming gas) because it was found to unite with chlorine to form $\text{CH}_2\text{Cl}.\text{CH}_2\text{Cl}$, an oily liquid, and the ethylene series is therefore also known as the *olefine series* or the *olefines*. The *general formula* of this homologous series is $\text{C}_n \text{H}_{2n}$. Isomerism is more characteristic in this series than in the corresponding paraffin series. For example, butylene shows the following isomeric forms:



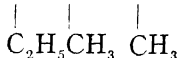
(2) The *acetylene series*, with a triple bond, forming the following *homologous series*:

Acetylene or ethine $\text{CH} \equiv \text{CH}$, methyl acetylene (allylene) or propine $\text{CH}_3.\text{C} \equiv \text{CH}$, ethyl acetylene or butine $\text{CH}_3.\text{CH}_2.\text{C} \equiv \text{CH}$, etc. The *general formula* of this series is $\text{C}_n\text{H}_{2n-2}$.

There may be other types of unsaturated hydrocarbons such as *diolefines* having two double bonds, e.g., butadiene $\text{CH}_2 = \text{CH}.\text{CH} = \text{CH}_2$, or *diacetylenes* having two triple bonds, e.g. dipropargyl $\text{CH} \equiv \text{C}.\text{CH}_2.\text{CH}_2.\text{C} \equiv \text{CH}$, or hydrocarbons with both double and triple bonds, e.g., vinyl acetylene, $\text{CH}_2 : \text{CH}.\text{C} \equiv \text{CH}$, and so on.

Nomenclature of Unsaturated Hydrocarbons.—According to the international system, the *olefines* are denoted

by the suffix *-ene*. The double bond is represented by the Greek letter *delta* (Δ) and the position of the double bond is given by the number of the carbon atom from which it begins. Thus, $\text{CH}_3.\text{CH}=\text{CH}.\text{CH}_2.\text{CH}_3$ will be called Δ 2-pentene, $\text{CH}_3.\text{CH}=\text{C}-\text{CH}-\text{CH}.\text{CH}_3$ will be called 4:5-dimethyl-



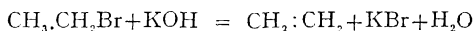
3-ethyl- Δ 2-hexene, and so on. *Diolefines* are denoted by the suffix *-diene* and the compound $\text{CH}_2=\text{CH}.\text{CH}=\text{CH}_2$ would be called Δ 1:3-butadiene. In the case of *acetylenes*; the international classification adopts the suffix *-ine*. Thus acetylene $\text{CH}\equiv\text{CH}$ is called ethine, allylene $\text{CH}_3.\text{C}\equiv\text{CH}$ is called propine, diacetylene $\text{CH}\equiv\text{C}.\text{C}\equiv\text{CH}$ is called butadiene, and so on.

Ethylene, Ethene, Olefiant Gas, C_2H_4 , $\text{CH}_2=\text{CH}_2$

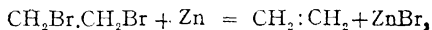
Occurrence.—It occurs in coal gas to the extent of about 3 per cent by volume and the luminosity of a coal gas flame is partly due to this gas. It is found in the natural gas of some petroleum wells and also in the gases from coke ovens and in wood gas.

Synthesis and Preparation.—

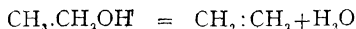
(1) By dropping ethyl bromide into a boiling strong alcoholic solution of caustic potash:



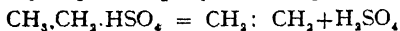
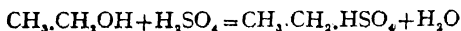
(2) By heating an alcoholic solution of ethylene dibromide with granulated zinc.



(3) By the action of dehydrating agents like concentrated sulphuric acid or concentrated orthophosphoric acid on ethyl alcohol:



In the case of sulphuric acid, ethyl hydrogen sulphate is first formed which at a higher temperature either alone or in the presence of sulphuric acid decomposes into ethylene liberating the acid:



In the laboratory, a mixture of ethyl alcohol with about six times its weight of concentrated sulphuric acid is taken in a round bottomed flask containing a little dry sand to prevent frothing (Fig. 23). The flask is provided with a dropping funnel containing a mixture of one part of alcohol and two parts of concentrated sulphuric acid. A thermometer dips into the liquid in the flask and the delivery tube leads to a series of wash bottles.

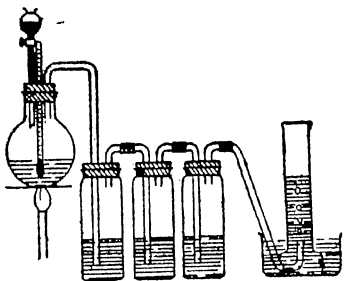


FIG. 23.

The liquid in the flask is heated over a sand bath to about 160°C and when a steady stream of gas is evolved the mixture in the tap funnel is allowed to fall drop by drop. The gas, which may contain as impurities vapours of ethyl alcohol and ether, is bubbled through wash bottles containing water and concentrated sulphuric acid and then through a concentrated solution of KOH to remove CO_2 and SO_2 likely to be formed during the process and it is finally collected over water.

The impurities formed by the use of conc. sulphuric acid such as CO_2 and SO_2 can be avoided and a purer gas obtained by taking *syrupey phosphoric acid* (a concentrated solution of orthophosphoric acid, H_3PO_4 , of sp. gr. 1.75) in place of conc. H_2SO_4 , and heating the liquid between 200° — 220°C .

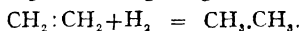
(4) On a large scale, the above process is modified by passing alcohol vapour through heated iron tubes containing syrupey phosphoric acid soaked in pumice stone.

Large quantities of ethylene are used for the manufacture of the poison gas known as mustard gas (see p. 133) or synthetic drugs like novocaine (pp. 351—52).

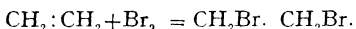
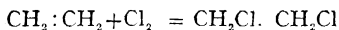
Properties, Reactions and Uses—

Ethylene is a light, colourless gas with a slight sweetish odour. When inhaled it produces anæsthesia and a mixture of ethylene with about ten per cent of oxygen is sometimes used as an anæsthetic. It is very slightly soluble in water but more readily in alcohol and ether. Liquefied ethylene boils at -105°C . Ethylene burns with a light luminous flame and forms an explosive mixture with air or oxygen.

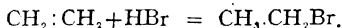
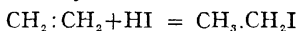
Being an unsaturated compound it shows great reactivity and yields various *additive compounds*. Thus, in presence of platinum black as catalyst it combines with *hydrogen* even at room temperature giving ethane:



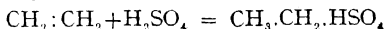
Ethylene combines directly with *chlorine* or *bromine* (but less readily with iodine dissolved in alcohol) to form ethylene dichloride (*Dutch Liquid*) and ethylene dibromide respectively:



With *halogen acids* ethylene gives alkyl halides. It combines most readily with HI and least with HCl:

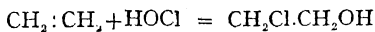


Concentrated or fuming *sulphuric acid* absorbs ethylene forming ethyl hydrogen sulphate or sulphovinic acid:

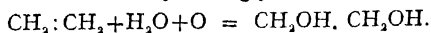


This reaction is utilized for removing unsaturated hydrocarbons from saturated ones and even for estimating them.

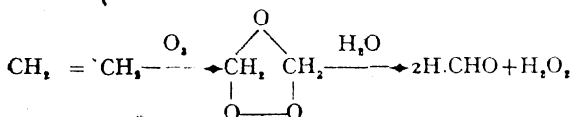
Hypochlorous acid combines with ethylene giving ethylene chlorohydrin, a colourless liquid used in the preparation of the local anæsthetic novocaine:



A dilute acid or alkaline solution of *potassium permanganate* is decolorized by ethylene, the latter being oxidized to the dihydric alcohol ethylene glycol:



With ozone ethylene forms an ozonide, the ozone molecule being attached at the double bond. These ozonides are easily decomposed by dilute acids into aldehydes and H_2O_2 , and the molecule is broken up at the double bond. A study of the decomposition products enables us to determine the position of the double bond in the molecule:



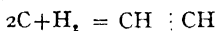
Ethylene is used for ripening green fruits like apples, oranges, bananas, etc. Its use in the preparation of mustard gas and synthetic drugs and also as an anæsthetic has been already mentioned.

Acetylene, Ethine, C_2H_2 , $\text{CH}\equiv\text{CH}$

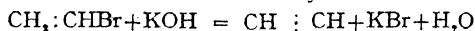
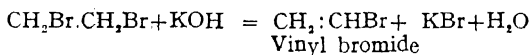
Occurrence.—It occurs in coal gas to the extent of about 0.06 per cent and is formed by the incomplete combustion of coal gas. Thus when a Bunsen burner 'strikes back', i.e., when the coal gas burns at the base, the issuing gas may contain from 0.6 to 0.8 per cent of acetylene.

Synthesis and Preparation.

(1) It has been synthesized directly from the elements by passing an electric arc between carbon poles in an atmosphere of hydrogen:

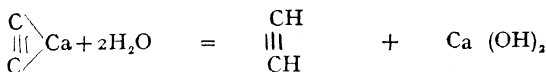
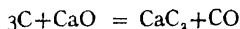


(2) *Pure acetylene* can be obtained by heating ethylene dibromide with an alcoholic solution of KOH, the reaction taking place as follows:



(3) *In the laboratory* it is easily prepared by the action of water on calcium carbide. The *calcium carbide* used for this purpose is made on a large scale by heating a mixture

of coke and lime at a very high temperature in an electric furnace and is found as greyish lumps:



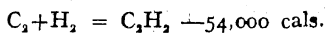
This method of preparation is of special interest since an organic compound is being synthesized directly from the carbon atom without the intervention of any life process, and acetylene can serve as the starting material for many other organic compounds of more complex nature.

A layer of sand is placed at the bottom of a conical flask to prevent local heating and cracking of the flask and small lumps of calcium carbide are placed over the sand. Water is allowed to drop slowly from a tap funnel and the gas evolved is passed through a wash bottle and then collected over water. The acetylene obtained from calcium carbide contains *impurities* like phosphine, sulphuretted hydrogen and ammonia and these are removed by an acidified solution of copper sulphate placed in the wash bottle.

Properties, Reactions and Uses—

It is a light colourless gas. When pure it has an agreeable, slightly ethereal odour but the gas obtained from calcium carbide has an unpleasant garlic-like odour due to the presence of impurities. Liquefied acetylene is a colourless liquid boiling at -82°C . Water dissolves about the same volume of acetylene at 18° and absolute alcohol or glacial acetic acid dissolves about six volumes of the gas at 18°C . One volume of acetone dissolves at 15° about 25 volumes of acetylene at one atmospheric pressure and about 300 volumes of the gas at 12 atmospheres.

Acetylene is an *endothermic substance*, i.e., it is formed from its elements with absorption of heat, the *heat of formation* being about $-54,000$ calories:

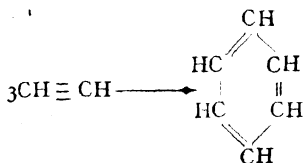


It is, therefore, a comparatively unstable compound and decomposes into its elements with explosive violence due to

the liberation of this large store of heat energy. Liquid acetylene or the compressed gas is easily decomposed by heat or detonation and may thus cause serious accidents due to explosions whereas a solution of the gas in acetone can be used with safety. The gas is, therefore, stored under pressure in strong iron cylinders which are filled with porous briquettes soaked in acetone.

When mixed with air or oxygen acetylene forms an explosive mixture. It usually burns with luminous, sooty and a very hot flame. it is used for lighting purposes (*e.g.*, in acetylene lamps used in bicycles or in domestic lighting) owing to its high illuminating power. A mixture of oxygen and acetylene used in the *oxyacetylene* blowpipe yields a very hot flame which is employed in cutting steel plates and for welding purposes.

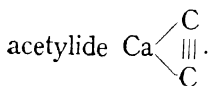
When acetylene is passed through an iron tube heated to dull redness it is partially converted into benzene:



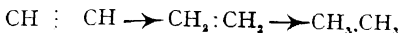
This is a typical example of the transformation of an open chain *aliphatic* hydrocarbon into an *aromatic* hydrocarbon with a ring structure, as well as the synthesis of an aromatic compound from its elements, acetylene being obtained from carbon and hydrogen. Again, we find that acetylene C_2H_2 has been converted into another compound having the same empirical formula but with a multiple molecular weight $(\text{C}_2\text{H}_2)_3$. This union of two or more molecules of one organic compound to form a more complex compound having a multiple molecular weight is known as *polymerization* (Gk. *polus*—many, *meros*—part). Here benzene is called a *polymer* of acetylene. The tendency to polymerize is found to be fairly common amongst unsaturated compounds.

Acetylides—

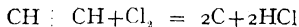
The two hydrogen atoms of acetylene are replaceable by metals like Cu, Ag, Hg, Na, etc., giving metallic derivatives known as *acetylides*. Thus when the gas is passed through ammoniacal solutions of cuprous chloride or silver nitrate, a reddish or chocolate brown precipitate of *cuprous acetylide* C_2Cu_2 or a yellowish white precipitate of *silver acetylide* C_2Ag_2 are obtained. In these reactions it acts as a dibasic acid and compounds like Na_2C_2 and $NaHC_2$ are also known. The dry copper, silver or mercury acetylides are highly explosive. The precipitate of the red cuprous acetylide serves as a very *delicate test* for acetylene and also for the separation of acetylene from other gases. Cuprous acetylide is decomposed by warming with dilute hydrochloric acid regenerating acetylene and *pure acetylene* can be obtained in this way. Calcium carbide may also be considered as calcium

*Additive Compounds*—

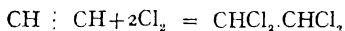
Like all unsaturated hydrocarbons acetylene forms various *additive compounds*. Thus, in the presence of catalysts like platinum black or finely divided nickel, acetylene is reduced by *hydrogen* giving first ethylene and then ethane:



With *chlorine* under ordinary conditions acetylene reacts with explosive violence giving C and HCl:

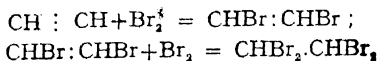


When the reaction takes place in presence of some diluent like kieselguhr, *acetylene tetrachloride* or tetrachlorethane is formed:

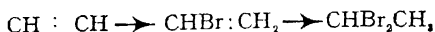


Acetylene tetrachloride is a non-inflammable liquid, technically known as *westron*, and is used in industry as a solvent for fats, rubber, cellulose acetate, etc. It is now used extensively in the manufacture of non-inflammable cinematographic films. It has also found use as an insecticide. It

has been known to have caused fatal poisoning by inhalation among the industrial workers. With *bromine*, acetylene gives first acetylene dibromide and then acetylene tetrabromide:

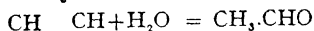


With *hydrobromic acid*, acetylene gives vinyl bromide and then ethylidene bromide:



In the second reaction, it may be observed that *the halogen atom unites with the carbon atom which is poorer in hydrogen: this is known as Markownikoff's Rule.*

In presence of catalysts, acetylene combines with water giving acetaldehyde:



Commercial acetaldehyde is prepared by passing acetylene gas through warm dilute sulphuric acid containing some mercuric sulphate which acts as a catalyst. The acetaldehyde is further oxidized to acetic acid and thus forms a source of synthetic acetic acid

CHAPTER VII

HALOGEN DERIVATIVES OF THE HYDROCARBONS

We have already seen that a *saturated hydrocarbon* like *methane* is acted upon by chlorine giving *substitution products* such as methyl chloride CH_3Cl , methylene chloride CH_2Cl_2 , chloroform CHCl_3 and carbon tetrachloride CCl_4 . Similarly, bromine would give methyl bromide CH_3Br , methylene bromide CH_2Br_2 , etc. Iodine substitution products such as CH_3I , CH_2I_2 , CHI_3 and CI_4 are also known although they are formed indirectly.

Ethane would likewise yield substitution products such as ethyl chloride $\text{CH}_3\text{CH}_2\text{Cl}$, ethylidene chloride CH_3CHCl_2 , tetrachlorethane $\text{CHCl}_2\text{CHCl}_2$, hexachlorethane CCl_3CCl_3 , etc.

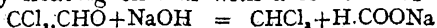
Unsaturated hydrocarbons, on the other hand, yield halogen derivatives by addition, such as dichlor-ethylene $\text{CHCl}:\text{CHCl}$, acetylene tetrabromide $\text{CHBr}_2\text{CHBr}_2$, etc., from acetylene; ethylene dibromide $\text{CH}_2\text{Br}.\text{CH}_2\text{Br}$, ethyl iodide $\text{CH}_3\text{CH}_2\text{I}$, etc., from ethylene, and so on.

Some of the halogen derivatives of hydrocarbons, such as chloroform, iodoform, tetrachlorethylene, etc., are used in medicine; some, for instance, carbon tetrachloride, ethylene dichloride (or dichloroethane) $\text{CH}_2\text{Cl}.\text{CH}_2\text{Cl}$, tetrachlorethane $\text{CHCl}_2.\text{CHCl}_2$, dichlorethylene $\text{CHCl}:\text{CHCl}$, trichlorethylene $\text{CCl}_2:\text{CHCl}$, etc., are used as insecticides and fumigants, ethylene dichloride being particularly useful against weevils in food-grains; others, such as carbon tetrachloride, methyl bromide, ethylene dibromide (or dibromoethane) $\text{CH}_2\text{Br}.\text{CH}_2\text{Br}$, etc., are used as fire extinguishers, and most of the above compounds are extensively used as solvents for rubber, etc., in various industries.

Chloroform, Trichloromethane, CHCl_3

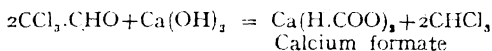
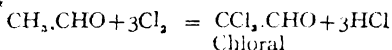
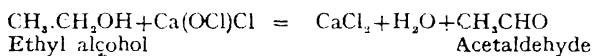
Preparation.

(I) *Pure chloroform* for anæsthetic purpose is best prepared by heating chloral with a solution of caustic soda:



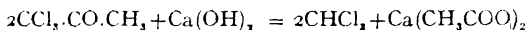
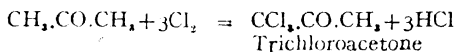
(2) Chloroform is prepared in the laboratory or on a large scale by the action of bleaching powder upon ethyl alcohol or acetone.

In the case of *ethyl alcohol* the chlorine liberated from the bleaching powder first converts the alcohol into chloral by simultaneous oxidation and chlorination. The lime present in the bleaching powder reacts with chloral to form chloroform and calcium formate.



The reactions shown in the above equations represent only a portion of the different stages of the production of chloroform. They have been discussed further in connection with chloral (see p. 117).

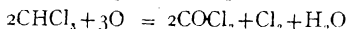
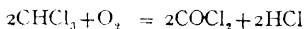
In the case of *acetone* the reaction proceeds more readily and it is, therefore, preferred to ethyl alcohol in the laboratory. The acetone is chlorinated into trichloroacetone which is then decomposed by the lime into CHCl_3 and Ca-acetate.



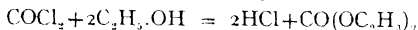
Chloroform prepared from acetone is likely to contain mono- and dichloro-acetones which are poisonous and thus render it unsuitable for anæsthesia.

To prepare chloroform *in the laboratory*, about 100 grams of bleaching powder are made into a paste with about 300 c.c. of water and taken in a large round bottomed flask. About 20 grams of acetone (or the same amount of alcohol, free from fusel oil) are added and the mixture is gradually heated on a sand bath. The flask is connected with a condenser and the chloroform is distilled over. The distillate is taken in a separating funnel, washed with dilute caustic soda and then with distilled water. It is then dried with anhydrous calcium chloride and purified by redistillation.

Properties.—Chloroform is a colourless liquid with a pleasant odour and a burning sweetish taste. It is a heavy, volatile liquid having a specific gravity of 1.489 at 20°. Pure chloroform has a boiling point of 61.2° (760 m.m.). It is almost insoluble in water, 100 grams of water dissolving only 0.822 gram of chloroform at 20° to which it imparts a sweetish taste. It is miscible with absolute alcohol, ether, petroleum ether and benzene. It is not inflammable but burns with a sooty flame if mixed with some alcohol. When exposed to light and air, especially in presence of moisture, it is partially oxidized and gives rise to the poisonous gas *phosgene* or carbonyl chloride COCl_2 and HCl together with some free chlorine.



All these impurities are dangerous for anæsthetic purpose. To avoid these reactions medicinal chloroform is mixed with 1 to 2 per cent by volume of anhydrous alcohol and kept in a cool dark place in a well-stoppered, amber coloured bottle filled up to the stopper leaving no air space in the bottle. The alcohol not only inhibits the oxidation but also converts the carbonyl chloride into ethyl carbonate which is harmless:

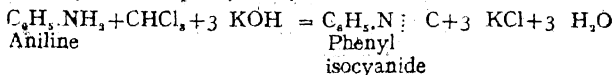


According to the B.P. the boiling point of chloroform containing 1 to 2 per cent of alcohol lies between 60° and 62° and has a specific gravity of 1.485 to 1.490 at 15.5°.

Uses.—Chloroform is a very good *solvent* for fats, oils, alkaloids, etc., and is widely used as such in industries and in chemical laboratories. Pure chloroform is used for producing *anæsthesia* in surgical operations and internally, as a *carminative* and *stomachic*. As an antiseptic it is used in biochemical work to prevent the growth of fungus or bacteria.

Reactions and Tests.

(i) On warming a drop of chloroform, a drop of aniline and a little alcoholic solution of caustic potash a disagreeable smell of phenyl isocyanide or phenylcarbylamine is obtained:



This is known as the *isocyanide test* or *carbylamine reaction*, and is a very delicate test for chloroform as well as for a primary amine (see p. 246).

(ii) Pure chloroform does not give any precipitate with silver nitrate since the chlorine atoms in the molecule cannot ionize. But if it is heated with an aqueous or better with an alcoholic solution of caustic potash, it is decomposed giving potassium formate and potassium chloride: $\text{CHCl}_3 + 4\text{KOH} = \text{H.COOK} + 3\text{KCl} + 2\text{H}_2\text{O}$. If the solution is acidified with dilute nitric acid and AgNO_3 added, a white precipitate of AgCl is at once obtained and the silver formate formed is also gradually reduced to metallic silver.

(iii) Chloroform slightly reduces Fehling's solution.

The **impurities** likely to be present in chloroform are COCl_2 , HCl , Cl , aldehydes, and some mineral matters. These are therefore tested for in anaesthetic chloroform.

For other tests see toxicology of chloroform.

Tests for Impurities in Anaesthetic Chloroform.

(i) *Hydrochloric Acid and Chlorides*. Shake 10 c.c. of chloroform with 20 c.c. of distilled water and allow to separate; test 5 c.c. of the water with blue litmus and 5 c.c. with silver nitrate.

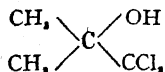
(ii) *Free chlorine*. Shake 10 c.c. of chloroform with 20 c.c. of distilled water and test 10 c.c. of the water with a solution of cadmium or potassium iodide and a few drops of starch solution; a blue colour if free chlorine is present.

(iii) *Test for phosgene, COCl_2* .—Add a pinch of phenyl hydrazine cinnamate to some of the chloroform; after 5 minutes add a drop of 1 per cent CuSO_4 ; a red-violet colour of diphenyl carbazide is formed if phosgene is present.

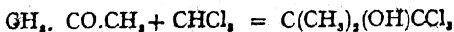
(iv) *Test for decomposition products*: 20 c.c. CHCl_3 are kept in the dark in a stoppered bottle with 15 c.c. conc. H_2SO_4 and 4 drops of a solution of formaldehyde and shaken frequently for $\frac{1}{2}$ hour; after an hour more the acid layer should not be more than slightly coloured.

Tests are also made for foreign chlorine compounds, aldehydes, residues, etc.

Chlorbutol, Chloretone, Trichloro-tertiary isobutyl alcohol



This compound is obtained by heating a mixture of chloroform and acetone with potassium hydroxide:



This is a colourless solid having a camphor-like odour and taste. When anhydrous it melts at 96° . It boils at 167° but is volatile at ordinary temperatures. It is easily soluble in ether, chloroform and

alcohol but less so in glycerine (1 in 10) or water (1 in 125). As a sedative it is used in sea-sickness, and as an antiseptic and analgesic it is used as a spray in its solution in liquid paraffin in order to relieve nasal catarrh.

Chloropicrin, Nitrochloroform, Trichloronitromethane, $C(NO_2)Cl_3$.

This is a pungent smelling liquid formed by the action of nitric acid on chloral. It is prepared by the action of bleaching powder on picric acid. It is almost insoluble in water; b.p. 112° ; sp. gr. 1.651 at 23° ; it has a strong irritant action on the eyes and mucous membrane and was used in the first Great War as a tear gas; minimum lethal concentration is 2.00.

N.B. Minimum lethal concentration shows relative toxicity on the basis of 10 minutes' exposure and is expressed in milligrams per litre.

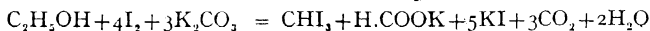
Bromoform, Tribromomethane, $CHBr_3$.

This is prepared by the action of bromine and a solution of sodium carbonate on acetone. It is a colourless liquid with a smell like chloroform and a sweetish taste. It boils at 150.5° (760 mm.) and melts at 7.8° and has a specific gravity of 2.9 at 20° . It is used as a sedative in whooping cough and as a solvent for the determination of molecular weights.

Iodoform, Triiodomethane, CHI_3 .

It is formed when ethyl alcohol (not methyl alcohol), acetone, aldehyde, etc., are warmed with iodine and an aqueous solution of an alkali. As iodoform is decomposed on boiling with caustic alkalies into a formate, no heat should be applied in preparing iodoform with NaOH or KOH in place of K_2CO_3 or Na_2CO_3 .

It can be prepared in the laboratory by warming an aqueous solution of K_2CO_3 with ethyl alcohol and iodine. The iodoform separates out as a crystalline powder which is filtered, washed with water and recrystallized from alcohol.



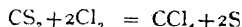
Iodoform is also prepared by the electrolysis of an aqueous alcoholic solution of KI and K_2CO_3 in presence of CO_2 at a temperature of $60-70^\circ$.

Iodoform crystallizes in lemon yellow shining hexagonal plates or star-shaped crystals (see Fig. 45, p. 467). It melts at 119° but volatilizes in steam and even at ordinary temperatures. It has a peculiar unpleasant odour and has a sp. gr. of 4.008 at 17° . It is insoluble in water but dissolves in

alcohol, ether and chloroform. It is used as an antiseptic for wounds. Owing, however, to its unpleasant odour and an irritant action on the skin it has been replaced by other compounds (see Iodol).

Carbon tetrachloride, Tetrachloromethane, CCl_4 .

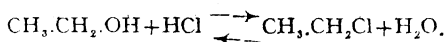
This is prepared on a large scale by the action of chlorine upon carbon disulphide in the presence of a catalyst, like iron filings, which acts as a *halogen carrier*.



It is a pleasant smelling colourless liquid with a burning taste. It boils at 76.74° (760 mm.) and has a sp. gr. of 1.594 at 20° . It is almost insoluble in water but dissolves easily in ether and absolute alcohol. It is very volatile and non-inflammable. It is used as a *solvent* for fats and oils in the *dry cleaning* of clothes and as a *fire-extinguisher* under the trade name *pyrene*. In medicine, it is used as an *anthelmintic* in tape-worm and hookworm infections, but owing to its higher toxicity, it is being replaced by tetrachlorethylene.

Ethyl chloride, $\text{CH}_3\cdot\text{CH}_2\text{Cl}$.

This is prepared by warming a saturated solution of hydrochloric acid in ethyl alcohol containing some anhydrous zinc chloride which acts as a dehydrating agent and prevents the reversible reaction:



It is a colourless volatile liquid with an ethereal smell and burning sweetish taste. It boils at 12.5° and has a sp. gr. of 0.92 at 0° . It is almost insoluble in water but is miscible with ether and absolute alcohol. It is used in minor surgical operations as a local anæsthetic in the form of a spray, the intense cold produced by its rapid evaporation causing anæsthesia of the part. Liquid ethyl chloride is also used for spraying perfumes.

Chlorine and fluorine derivatives of methane and ethane, e.g. CCl_2F_2 , $\text{CCl}_2\text{F}\cdot\text{CClF}_2$, $\text{CClF}_2\cdot\text{CClF}_2$, etc., have lately been introduced under the trade name of Freon and are used as refrigerants and insecticides.

Tetrachlorethylene, Perchlorethylene, CCl_2 : CCl_2 .

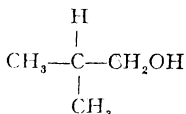
This is obtained as a bye-product in the commercial preparation of carbon tetrachloride from carbon disulphide and chlorine. It is a colourless liquid boiling at 121° and has a sp. gr. of 1.619 at 20° . It is used as an *anthelmintic* in the treatment of hookworm, threadworm, fluke and tapeworm infections.

CHAPTER VIII

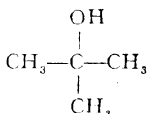
MONOHYDRIC ALCOHOLS

A. Saturated Monohydric Alcohols

If one atom of hydrogen in a saturated hydrocarbon like methane CH_4 is replaced by the monovalent radical —OH (hydroxyl), we get an alcohol known as methyl alcohol. Similarly, from ethane CH_3CH_3 we get the monohydric alcohol, ethyl alcohol $\text{CH}_3\text{CH}_2\text{OH}$. From propane $\text{CH}_3\text{CH}_2\text{CH}_3$ we can get two monohydric alcohols, *primary* propyl alcohol $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$ and *secondary* propyl alcohol $\text{CH}_3\text{CHOHCH}_3$. From n-butane $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_3$ we can get two alcohols, primary (n-) butyl alcohol $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$ and secondary (n-) butyl alcohol $\text{CH}_3\text{CH}_2\text{CHOHCH}_3$, and from iso-butane $\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_3$ we can get two alcohols, viz., *primary iso-butyl* alcohol and *tertiary iso-butyl* alcohol:



Primary iso-butyl alcohol



Tertiary iso-butyl alcohol

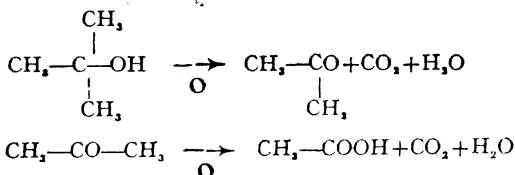
The monohydric alcohols are thus paraffins in which one atom of hydrogen has been replaced by a hydroxyl group. In a *primary alcohol* the hydroxyl group is attached to the end carbon atom and it contains the group $\text{—CH}_2\text{OH}$ but a *secondary alcohol* contains the group >CH.OH attached to two other carbon atoms and a *tertiary alcohol* contains the group —C.OH attached to three other carbon atoms.

The *behaviour on oxidation* is also quite different for the three types of alcohols. A *primary alcohol* $\text{R.CH}_2\text{OH}$ (where R represents an organic radical) is oxidized first to an aldehyde R.CHO , and then to an acid R.COOH (both

containing the same number of carbon atoms. Thus, the primary ethyl alcohol is oxidized first to acetaldehyde and then to acetic acid: $\text{CH}_3\text{CH}_2\text{OH} \xrightarrow{\text{O}} \text{CH}_3\text{CHO} \xrightarrow{\text{O}} \text{CH}_3\text{COOH}$.

A *secondary alcohol* is oxidized to a ketone containing the same number of carbon atoms. The ketone on further oxidation is broken up into an acid having a lower number of carbon atoms. Thus, the secondary alcohol isopropyl alcohol having three carbon atoms is oxidized first to acetone and then into acetic acid which contains two carbon atoms: $\text{CH}_3\text{CH}(\text{OH})\text{CH}_3 \xrightarrow{\text{O}} \text{CH}_3\text{COCH}_3 \xrightarrow{\text{O}} \text{CH}_3\text{COOH} + \text{CO}_2 + \text{H}_2\text{O}$

A *tertiary alcohol* is broken up on oxidation into ketones and acids both having a lower number of carbon atoms. Thus, tertiary butyl alcohol having four carbon atoms is oxidized to acetone (3C atoms) which breaks down further into acetic acid (2C atoms) and carbon dioxide:



Nomenclature of Monohydric Alcohols.

The classification into primary-, secondary- and tertiary alcohols according to their characteristic groups and that based on the hydrocarbon into normal- and *iso*- has been mentioned above.

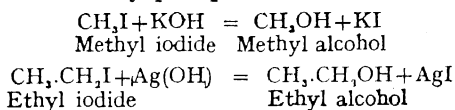
Another convenient nomenclature depends upon considering all the alcohols as derivatives of methyl alcohol CH_3OH which is called *carbinol*. Thus, ethyl alcohol $\text{CH}_3\text{CH}_2\text{OH}$ is methyl carbinol, iso-propyl alcohol $\text{CH}_3\text{CH}(\text{OH})\text{CH}_3$ is called dimethyl carbinol, and so on.

According to the international (Geheva) system of nomenclature, the suffix *-ol* is added to the corresponding hydrocarbon after dropping the ending *-e*. Thus methyl alcohol is methanol, ethyl alcohol is ethanol, n-propyl alcohol

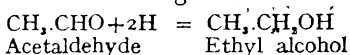
propan-1-ol, isopropyl alcohol is propan-2-ol, primary n-butyl alcohol $\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}_2\text{OH}$ is butan-1-ol, secondary-n-butyl alcohol $\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}(\text{OH})\cdot\text{CH}_3$ is butan-2-ol, primary isobutyl alcohol $\text{CH}_3\cdot\text{CH}(\text{CH}_3)\cdot\text{CH}_2\text{OH}$ is 2-methylpropan-1-ol, tertiary isobutyl alcohol $\text{CH}_3\cdot\text{C}(\text{OH})\cdot\text{CH}_3$ is 2-methylpropan-2-ol, and so on. CH_3

Some General Methods of Synthesis of Alcohols.

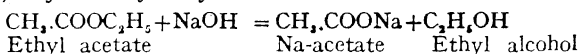
(1) By heating alkyl halides with dilute aqueous alkalis or with freshly precipitated moist silver oxide:



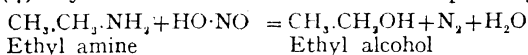
(2) By the reduction of aldehydes with nascent hydrogen, e.g., by the use of sodium amalgam and dilute sulphuric acid:



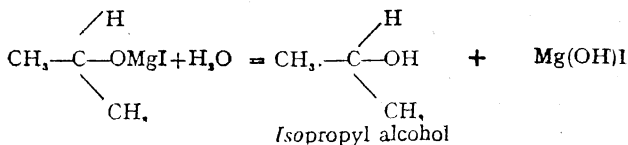
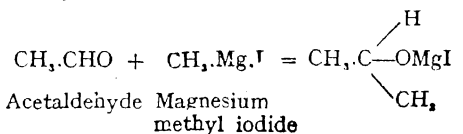
(3) By the hydrolysis of esters with dilute alkalis:

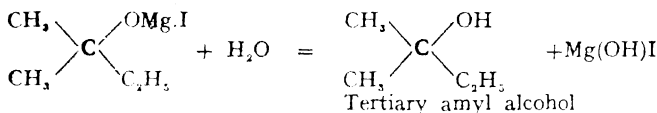
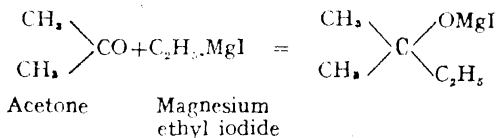


(4) By the action of nitrous acid on primary amines:



(5) By the action of *Grignard Reagents* on aldehydes or ketones; this method is specially suitable for the preparation of secondary and tertiary alcohols:





General Properties and Reactions of Alcohols.

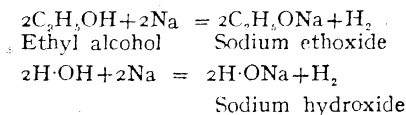
(i) Physical Properties.

The alcohols are all neutral in reaction and are lighter than water. The lower primary alcohols like methyl-, ethyl-, propyl-, butyl- and amyl alcohols are mobile colourless liquids, the middle ones are oily and the higher ones, from dodecyl alcohol $\text{C}_{11}\text{H}_{23}\cdot\text{CH}_2\text{OH}$ (m.p. 24°) upwards, are wax-like solids. The two lower ones, methyl alcohol and ethyl alcohol, are easily soluble in water but the solubility of the other alcohols decreases rapidly with the increase in molecular weight. The lower members have characteristic odours but the higher ones are almost odourless and tasteless like the paraffins. They have the general formula $\text{C}_n \text{H}_{2n+1} \text{OH}$. The *primary alcohols* form the following *homologous series*:

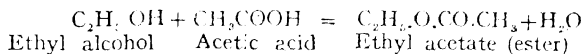
- $\text{H}\cdot\text{CH}_2\text{OH}$ methyl alcohol ($\text{CH}_3\cdot\text{OH}$).
- $\text{CH}_3\cdot\text{CH}_2\text{OH}$ ethyl alcohol ($\text{C}_2\text{H}_5\cdot\text{OH}$).
- $\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\text{OH}$ n-propyl alcohol ($\text{C}_3\text{H}_7\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_2\cdot\text{CH}_2\text{OH}$ n-butyl alcohol ($\text{C}_4\text{H}_9\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_3\cdot\text{CH}_2\text{OH}$ n-amyl alcohol ($\text{C}_5\text{H}_{11}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_4\cdot\text{CH}_2\text{OH}$ n-hexyl alcohol ($\text{C}_6\text{H}_{13}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_5\cdot\text{CH}_2\text{OH}$ n-heptyl alcohol ($\text{C}_7\text{H}_{15}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_6\cdot\text{CH}_2\text{OH}$ n-octyl alcohol ($\text{C}_8\text{H}_{17}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_7\cdot\text{CH}_2\text{OH}$ n-nonyl alcohol ($\text{C}_9\text{H}_{19}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_8\cdot\text{CH}_2\text{OH}$ n-decyl alcohol ($\text{C}_{10}\text{H}_{21}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_9\cdot\text{CH}_2\text{OH}$ n-undecyl alcohol ($\text{C}_{11}\text{H}_{23}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_{10}\cdot\text{CH}_2\text{OH}$ n-dodecyl alcohol ($\text{C}_{12}\text{H}_{25}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_{11}\cdot\text{CH}_2\text{OH}$ n-tridecyl alcohol ($\text{C}_{13}\text{H}_{27}\cdot\text{OH}$).
- $\text{CH}_3\cdot(\text{CH}_2)_{14}\cdot\text{CH}_2\text{OH}$ cetyl alcohol ($\text{C}_{17}\text{H}_{35}\cdot\text{OH}$).
- $\text{CH}_3(\text{CH}_2)_{26}\cdot\text{CH}_2\text{OH}$ melissyl alcohol ($\text{C}_{36}\text{H}_{61}\cdot\text{OH}$).

(ii) Chemical Reactions:

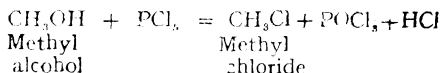
Metallic sodium acts on an alcohol and replaces the hydrogen atom of the hydroxyl group, forming *alcoholates*; the reaction is similar to that with water:



Alcohol combines with organic acids forming a class of compounds known as *esters*:



Phosphorus pentachloride reacts with alcohols and replaces the OH group by Cl:

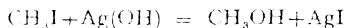


Methyl Alcohol, Methanol, Carbinol, Wood Spirit,
 CH_3OH

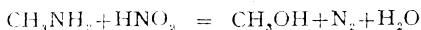
Occurrence.—It does not occur in the free state in nature but is found as an ester in the essential oils of many plants. Thus it is found as methyl ester of salicylic acid in the oil of wintergreen obtained from the herb *Gaultheria procumbens* Linn., as methyl ester of anthranilic acid in the oil of tangerine obtained from the peel of the fruits of *Citrus japonica* Thunb., as methyl cinnamate in the oil of *Alpinia galanga* Willd., etc. The alkaloid cocaine found in the leaves of *Erythroxylon coca* Lam., is a methyl ester of benzoyl ecgonine (see cocaine).

Synthesis:

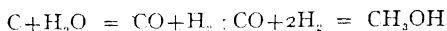
(1) By the action of moist silver oxide on methyl iodide:



(2) By the action of nitrous acid on methyl amine:



(3) *On a large scale* it is synthesized by heating carbon monoxide with hydrogen under a pressure of 300 to 600 atmospheres at 300 to 400°C in presence of suitable catalysts such as chromium oxide, etc. Since the mixture of CO and H_2 , known as *water gas* is obtained by passing steam over red hot coke, this method is really a synthesis from its elements:



The alcohol thus obtained is purified by distillation.

Preparation from Dry Distillation of Wood.—Methyl alcohol is generally prepared on a large scale by the *destructive distillation of wood*. Dry wood (beech, birch, pine, eucalyptus, etc.) is heated in large iron retorts to which there is no access of air and the following products of distillation are collected after passing through condensers: (1) The gas given off, known as *wood gas*, consists of hydrogen, methane and other hydrocarbons, carbon monoxide, etc. and is used as fuel for heating the retorts. (2) A reddish-brown *aqueous liquid* and (3) some tar known as *wood tar* collect in the distillate. (4) *Wood charcoal* remains behind in the retorts. The aqueous distillate, which is acid in reaction and known as *pyroligneous acid* forms about 45 per cent of the weight of the wood and this is separated mechanically from the tar. The wood tar, which contains paraffins, naphthalene, phenol, guaiacol, etc., and possesses antiseptic properties, is used as a preservative for timber.

The pyroligneous acid owing to its content of acetic acid is also known as crude *wood vinegar*. It contains from 8 to 10 per cent of acetic acid, from 2 to 4 per cent of methyl alcohol, about 0.5 per cent of acetone, and traces of esters and other substances. The liquid is neutralized with milk of lime $\text{Ca}(\text{OH})_2$ in order to fix the acetic acid as non-volatile calcium acetate and distilled. The distillate which consists of a dilute solution of methyl alcohol and acetone together with other volatile components is treated with quicklime and distilled fractionally. The product obtained contains from 70 to 80 per cent of methyl alcohol with 10 to 20 per cent of acetone together with other impurities and is known as *commercial wood spirit* or *wood naphtha*.

Purification of Methyl Alcohol

The commercial wood spirit can be purified further by treatment with quicklime followed by careful fractional distillation. But it is not possible to get either absolute methyl alcohol or pure acetone by continuing this process. To obtain pure methyl alcohol free from acetone one of the following methods is used: (1) The alcohol is treated with dry *chlorine* which converts the acetone into high boiling chloro-derivatives, mainly trichloro-acetone, and the liquid distilled fractionally. (2) The alcohol is treated with *anhydrous calcium chloride* when a crystalline compound $\text{CaCl}_2 \cdot 4\text{CH}_3\text{OH}$ is formed; the acetone (b.p. 56°C) is removed by draining and heating and the

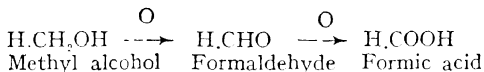
crystalline compound which is not decomposed at a temperature below 100°C is then decomposed by boiling with water, and the alcohol distilled over; the distillate is dehydrated by treatment with quicklime and redistilled. (3) The alcohol is treated with *anhydrous* oxalic acid when a solid dimethyl oxalate $\text{CH}_3\text{OOC}\cdot\text{COOCH}_3$, m.p. 54° , b.p. 153° , is formed; this is washed with water to remove the acetone and then distilled with KOH when methyl alcohol is obtained; this is treated with quicklime and re-distilled.

Properties and Reactions.

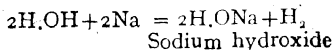
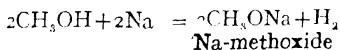
Methyl alcohol is a mobile colourless liquid neutral in reaction and boils at 64.7° (760 mm.). It has a sp. gr. of 0.791 at 20° . It mixes with water in all proportions. It has a peculiar odour and a burning taste. It produces intoxication as with ethyl alcohol but is extremely poisonous and is liable to produce blindness and even death (see Toxicology, pp. 470-71).

Methyl alcohol cannot be dehydrated by anhydrous calcium chloride since it combines with it to form $\text{CaCl}_2\cdot 4\text{CH}_3\text{OH}$ which crystallizes as hexagonal plates. The alcohol present in such compounds may be termed *alcohol of crystallization* since it is similar to the water of crystallization of the compound $\text{CaCl}_2\cdot 6\text{H}_2\text{O}$. With MgCl_2 a similar compound $\text{MgCl}_2\cdot 6\text{CH}_3\text{OH}$ is formed.

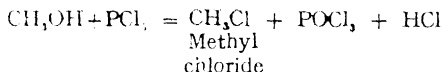
Methyl alcohol burns with a pale blue non-luminous flame. Its vapour forms an explosive mixture with air or oxygen. As a primary alcohol, it is oxidized first to an aldehyde, known as formaldehyde, and then to an acid called formic acid:



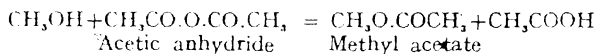
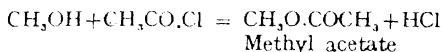
The typical reactions of the hydroxyl group (OH) present are given by metallic sodium, phosphorus pentachloride, acetyl chloride and acetic anhydride. Thus metallic sodium replaces the H atom of the OH group, produces sodium methoxide and liberates hydrogen gas; the reaction is similar to that with water:



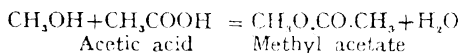
Phosphorus pentachloride replaces the OH group by chlorine:



Acetyl chloride or acetic anhydride replaces the H atom of the OH group by *acetyl group* ($\text{CH}_3\text{CO}-$):



When warmed with acetic acid, methyl alcohol gives the ester methyl acetate:



Uses of Methyl alcohol.

Methyl alcohol is used to prepare *formalin* which is widely used as an antiseptic and preservative (see p. III). The use of the alcohol or its derivatives for the synthesis of various dyes and chemicals is due to the fact that they serve as the source of the methyl group. The alcohol is used as a solvent for varnishes, lacquers, etc., and as a fuel in motor cars. Owing to its poisonous properties the impure alcohol, wood spirit or wood naphtha, is used in Europe and America to prepare what is popularly known as *methylated spirit*.

Tests.

(1) *Methyl Salicylate Test:* To 3 or 4 drops of methyl alcohol add 2 drops of conc. H_2SO_4 and a pinch of salicylic acid; warm on a water bath and pour the liquid into a dilute solution of sodium carbonate; the pleasant odour of oil of wintergreen is perceived.

(2) Pure methyl alcohol is distinguished from ethyl alcohol by the fact that it *does not give the iodoform reaction*.

(3) *Oxidation Tests:*

(i) Take in a test tube about 5 c.c. of a saturated solution of sodium dichromate and 2 c.c. of dilute sulphuric acid (1:1) and add about 2 c.c. of the alcohol or its aqueous solution. Boil under reflux, cool, dilute with the same volume of water, distil and collect about 1 c.c. of the distillate in a little water in the receiving test

tube. To a portion of the distillate add (a) a dilute solution of mercuric chloride and warm; a white precipitate of mercurous chloride is obtained; (b) to another portion add an ammoniacal solution of AgNO_3 and warm; a silver mirror or a black precipitate is formed. These reactions are due to the formation of formic acid.

(ii) The most delicate method for testing methyl alcohol consists in *oxidizing it to formaldehyde* and then identifying the latter by various well known tests some of which are given here:

Take 5 c.c. of the solution suspected to contain methyl alcohol in a wider test tube, add 2.5 c.c. of 2 per cent KMnO_4 solution, 0.2 c.c. of conc. H_2SO_4 and mix carefully by shaking. Allow to stand for 2—3 minutes and then add 0.5 c.c. of 10 per cent oxalic acid solution and mix carefully; the colour of the permanganate gradually disappears. When the solution is of sherry-yellow colour, add 1 c.c. of conc. H_2SO_4 , mix and cool (Denige). The colour is completely discharged and the mixture now contains formaldehyde, the oxidation product of methyl alcohol. With this colourless mixture carry out the following tests:

(a) To about 3 c.c. of this mixture add half the volume of Schiff's reagent, mix and allow to stand—a blue-violet colour develops in the course of a minute or two, the depth of the colour depending upon the proportion of methyl alcohol in the original liquid taken for examination. Except in highly diluted solutions the colour usually attains its maximum depth within 15 minutes (Denige's Test).

This test is very *useful for detecting even 0.1 per cent methyl alcohol present in ethyl alcohol*, if the quantities of the reagents as described in the test are strictly adhered to.

N.B.—Ethyl alcohol is oxidized to acetaldehyde which also reacts with Schiff's reagent but in the presence of excess of H_2SO_4 acetaldehyde does not give any coloration while the formaldehyde does. Denige's test is based on this property of acetaldehyde.

(b) To about 1 c.c. of the mixture add 5 c.c. of conc. H_2SO_4 , mix and cool. Add a drop of this solution to a small amount (about 1 mg.) of morphine or its salt on a white plate—a beautiful violet or blue-violet colour appears immediately (Marquis).

(c) To another portion of 1 c.c. add 5 c.c. of conc. H_2SO_4 , mix and cool as before. Dissolve a pinch of morphine or its salt in 2 c.c. conc. H_2SO_4 in another test tube by shaking. Mix the two solutions together—a blue-violet colour develops in a few seconds (Mannich).

(d) To another portion of about 2 c.c. add about 3 c.c. of ordinary bazar milk, mix and cool. Gently layer at the bottom of the milk about 3 c.c. of conc. H_2SO_4 to which a small drop of dilute FeCl_3 solution was added—a beautiful purple ring immediately appears at the junction of the two liquids (Hehner). This test was originally devised by Hehner for detection of formaldehyde added to stale milk as a preservative.

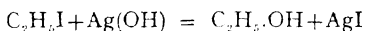
N.B.—*Schiff's reagent* is prepared as follows: Dissolve 0.2 gram of basic fuchsine in 120 c.c. of hot distilled water. Cool the solution, add a solution of 2 grams of anhydrous sodium sulphite in 20 c.c. of distilled water, and then add 2 c.c. of concentrated HCl and mix. Dilute the solution with water and make up to 200 c.c. and allow to stand for one hour before use. The reagent should be prepared every few days as it loses its potency on keeping. It, however, keeps fairly well if stored in a refrigerator.

Ethyl Alcohol, Alcohol, Ethanol, Methyl Carbinol, Spirit of Wine, $\text{CH}_3\text{CH}_2\text{OH}$.

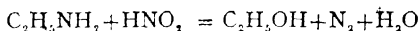
Occurrence.—It is stated to be present as an ester in some oils of Eucalyptus. The presence of the free alcohol in some fruit juices, e.g., grapes, is due to the fermentation of the sugar present. It is found in the urine, blood, cerebro-spinal fluid and also in the tissues of those who drink heavily (see pp. 466—469).

Synthesis.

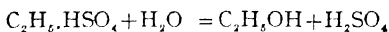
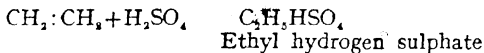
(1) By the action of moist silver hydroxide on ethyl iodide:



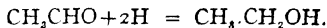
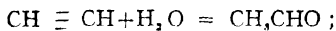
(2) By the action of nitrous acid on ethylamine:



(3) By the action of ethylene on fuming sulphuric acid, ethyl hydrogen sulphate is formed. When the latter is boiled with water ethyl alcohol is obtained.



(4) As stated before, acetaldehyde can be obtained from acetylene by the action of dilute sulphuric acid in presence of a catalyst. When acetaldehyde is reduced by hydrogen in presence of finely divided nickel as catalyst alcohol is formed:



Preparation of Ethyl Alcohol on a large Scale.

Starting Materials.—Alcohol is prepared by the fermentation of some sugars, chiefly glucose. These fermentable sugars are obtained from various sources. Thus the non-crystallizable residue, known as *molasses* obtained during the manufacture of cane sugar either from beet root or from sugar cane, contains on an average about 50 per cent of fermentable sugars. These are hydrolyzed and fermented with the help of yeast to produce alcohol. The *flowers of Bassia latifolia* Roxb., known as Mahua flowers, which contain up to about 60 per cent (calculated on dry weight) of fermentable sugars, serve as a good source of alcohol in this country where the tree grows fairly wild. Even the waste *sulphite liquors* of the wood pulp industry which contain about 2 per cent of fermentable sugars are utilized for the preparation of alcohol. The substances rich in *starch* such as potato and cereals such as rice, wheat, maize, barley, rye, etc., serve as important starting materials since starch can be easily hydrolyzed and fermented with the help of enzymes present in yeasts and moulds. Even materials rich in *cellulose*, which can be hydrolyzed to glucose by the action of sulphuric acid at a suitable temperature and high pressure, are utilized for the preparation of alcohol, and saw dust, wood or wood pulp has also been used for this purpose.

Hydrolysis and Fermentation of Starchy Materials.

Certain living cells such as yeasts, moulds or bacteria are known to secrete various *organic catalysts* and these are known as *ferments* (Latin, *fervere*—to boil) or more correctly *enzymes* (Gk. *en*—in, *zyme*—yeast). The decomposition of sugars by enzymes present in yeast or other living cells with the formation of alcohol is known as *alcoholic fermentation*. The name fermentation came from the fact that during this process, especially in the case of alcoholic fermentation, the liquid has the appearance of boiling owing to the evolution of bubbles of carbon dioxide although there is scarcely any rise of temperature. *Fermentation* is, however, a general term meaning the breaking down of higher organic compounds into lower ones by the action of enzymes found in living cells although there may not be any production of CO_2 gas.

Thus, we shall come across other types of fermentation such as *lactic acid fermentation*, *butyric acid fermentation*, *butyl alcohol—acetone fermentation*, etc.

The species of yeast used by brewers for the production of alcohol is known as *Saccharomyces cerevisiae*. Yeast is a microscopic single celled fungus which is more or less ovoid



FIG. 24.

in shape (Fig. 24) and measures 8 to 9 μ in diameter ($1 \mu = 0.001 \text{ mm.}$). When allowed to grow in a sugar solution it rapidly multiplies by budding at an enormous rate. Yeast secretes several enzymes, the more important of which for hydrolysis or alcoholic fermentation are *maltase*, *invertase*

and *zymase*, the two former for hydrolysis of disaccharides (malt sugar and cane sugar) into monosaccharides (grape sugar and fruit sugar) and the third for the production of alcohol from sugar. The above species of yeast can ferment glucose, fructose and mannose readily while galactose is only slowly acted upon and lactose and pentoses are not attacked at all. Yeast grows most rapidly between 25° and 40° and its action almost stops when the percentage of alcohol reaches about 14 per cent.

Manufacture of Alcohol from Potato Starch.—The crushed potatoes are heated with steam to about 130° to 140° under a pressure of about 3 atmospheres. The *mash*, containing the starch granules liberated from the cells, is then treated with finely powdered *malt* (p. 83) at about 60° . The enzyme *diastase* of the malt converts the starch into dextrin and maltose and the enzyme *maltase* also present in the malt converts the maltose into glucose. The liquid thus obtained contains the sugars and malt extract and is called *wort*. This is cooled and treated with pure cultures of *yeast* and the action is allowed to go on at about 30° . The *maltase* of yeast converts any unchanged maltose into glucose and the *zymase* of yeast then ferments the glucose into alcohol and carbon dioxide. (When *molasses* is used, the *invertase* of yeast hydrolyzes the cane sugar into glucose and fructose which are then fermented by *zymase*.) After about 3 days the fermentation is complete and the liquid contains nearly 13 per cent of alcohol together with various other products

of fermentation mentioned below. The fermented liquid or "wash" as it is called is distilled in specially constructed *stills*, such as Coffey's still or Patent still, which not only distils the spirit but purifies or rectifies it at the same time in its fractionating column known as "rectifier." The spirit thus obtained contains 90 to 95 per cent by volume of alcohol and is known as *rectified spirit* or 'patent still spirit'.

Malt is prepared from barley grains. The whole grains are steeped in water for a while and allowed to swell and soften. They are then spread out on a warm floor and kept moist until small shoots appear. The germination is then stopped by moderate heat and the grains are brushed, the clean product being known as malt. During the germination of the barley grains the enzymes diastase and maltase are formed. Various *malt preparations* in medicinal use are prepared by mixing an extract of malt with other substances.

Amylase-process for the Manufacture of Alcohol.

On the Continent, the mash containing starch liberated from the cells of starchy materials is treated with certain moulds, e.g. a species of *mucor* (*Mucor racemosus*), in place of malt. A gram of the mould is said to be equivalent to about 3 tons of malt. The starch is converted into glucose and both the hydrolysis and fermentation can be done in one operation by adding yeast along with the mould.

In India, a similar method of fermentation of starch, usually rice starch, by moulds has been known to tribal and hill peoples from ancient times. They used to distil liquor illicitly but now it is permitted by Government under license and it is carried out extensively for the manufacture of an alcoholic beverage known as *Pachwai*, containing a maximum of 12 per cent of alcohol. The fermenting agent is called *bakhar* consisting of mucors and some wild yeasts made into balls with rice starch. The manufacturers sometimes add powdered *Datura seeds*, *Nux Vomica seeds* or *Aconite roots* to the *bakhar* with the false notion of increasing the intoxicating power of the beverage. The result of this practice sometimes proves disastrous to the consumers.

The yield of alcohol from starch varies very much, depending upon the technique of fermentation and distillation. The theoretical yield is about 56 per cent of the total weight of starch used but actually the yield does not exceed 48 per cent.

Mechanism of Alcoholic Fermentation. The fermentation of glucose by yeast with the formation of equivalent amounts of alcohol and carbon dioxide may be expressed simply as follows: $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$. But the reaction is not so simple since we know that several *by-products* are formed. Thus, besides the evolution of *carbon dioxide* and the formation of about 13 per

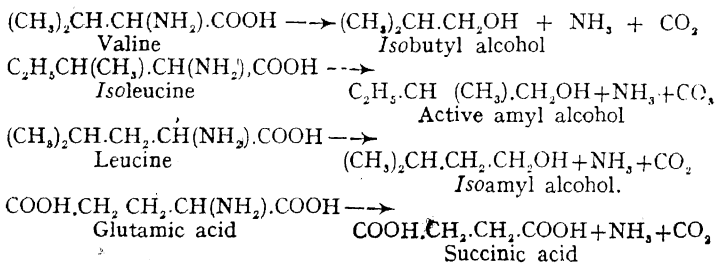
cent of *alcohol*, we get 3 to 4 per cent of *glycerol*, 0.1 to 0.7 per cent of higher alcohols or *fusel oil*, some acetic and *succinic acid* together with smaller amounts of *acetaldehyde*, *furfuraldehyde*, *pyruvic acid*, esters, etc.

Various *theories* have been advanced to account for these by-products and the one suggested by Meyerhof, which finds general acceptance, can be stated briefly as follows: Glucose is first converted into a hexose monophosphate and a hexose diphosphate. In the presence of the latter, which acts as a catalyst, the hexose monophosphate is broken up into glyceraldehyde phosphate $\text{CHO}.\text{CHOH}.\text{CH}_2\text{O}.\text{PO}_3\text{Na}_2$ which changes into α -glycerophosphate $\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{O}.\text{PO}_3\text{Na}_2$ and disodium phosphoglyceric acid $\text{COOH}.\text{CHOH}.\text{CH}_2\text{O}.\text{PO}_3\text{Na}_2$. The latter is hydrolyzed giving *pyruvic acid* which is then changed into *acetaldehyde* and *carbon dioxide*. The *glycerol* is formed by the hydrolysis of α -glycerophosphate. The *acetaldehyde* is reduced by glyceraldehyde phosphate to *ethyl alcohol* and the glyceryl phosphate is oxidized to phosphoglyceric acid.

Fusel Oil.

It is a mixture of several higher alcohols such as *n*-propyl alcohol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$, *iso* propyl alcohol $\text{CH}_3.\text{CHOH}.\text{CH}_3$, *n*-butyl alcohol $\text{CH}_3.\text{CH}_2.\text{CH}_2.\text{CH}_2\text{OH}$, *isobutyl* alcohol $(\text{CH}_3)_2\text{CH}.\text{CH}_2\text{OH}$, *tertiary* butyl alcohol $(\text{CH}_3)_3\text{C}.\text{OH}$, *n*-amyl alcohol $\text{CH}_3.(\text{CH}_2)_4.\text{CH}_2\text{OH}$, active amyl alcohol $\text{C}_2\text{H}_5.\text{CH}(\text{CH}_3).\text{CH}_2\text{OH}$, *isoamyl* alcohol $(\text{CH}_3)_2\text{CH}.\text{CH}_2.\text{CH}_2\text{OH}$, together with some other higher homologous alcohols and esters. The chief constituent is *isoamyl alcohol* which is also known as *fermentation amyl alcohol*.

The *fusel oil* is derived from the *amino acids* obtained from the proteins of the medium or from the autolysis of yeast cells. Thus *isobutyl* alcohol is probably derived from *valine*, active amyl alcohol from *isoleucine*, and *isoamyl* alcohol from *leucine*. The presence of *succinic acid* is also attributed to one of these amino acids known as *glutamic acid*:



The **rectified spirit** of the British Pharmacopœia should have a sp. gr. between 0.832 and 0.835 at 15.5°

and corresponds to about 90 per cent of alcohol by volume and about 86 per cent of alcohol by weight. The *absolute alcohol* of B.P., also known as *dehydrated alcohol*, should contain not less than 99.4 per cent by volume and 99.0 per cent by weight of alcohol and a sp. gr. between 0.7936 and 0.7967 at 15.5°.

Preparation of Absolute Alcohol and Anhydrous Alcohol.

—It has been found that rectified spirit containing 95.6 per cent of alcohol forms a constant boiling mixture or *azeotropic mixture* with a boiling point of 78.13° at 760 mm. It is not, therefore, possible to dehydrate this alcohol further by fractional distillation alone. *On a large scale*, the process known as the *azeotropic process* is utilized to dehydrate rectified spirit and it yields nearly 99.9 per cent of alcohol. The rectified spirit is mixed with a definite proportion of *benzene* and distilled. A constant boiling mixture of water, alcohol and benzene (b.p. 65°) first comes over, then another constant boiling mixture containing alcohol and benzene (b.p. 68.2°) distils over leaving behind pure alcohol which is obtained by further distillation. *In the laboratory*, however, one can prepare absolute alcohol by treating rectified spirit with freshly ignited *quicklime* (CaO) and redistilling. An alcohol containing 0.3 to 0.5 per cent of water can be thus obtained. To prepare perfectly *anhydrous alcohol*, the absolute alcohol can be treated with metallic calcium, or calcium carbide or magnesium amalgam and redistilled.

The term **proof spirit** is in common use in the excise department and originates from the old custom of testing the strength of a sample of alcohol by pouring it on to gunpowder and then igniting it. The most dilute alcohol which would ignite the gunpowder was called proof spirit. As laid down by the Act of Parliament, proof spirit should contain 49.24 parts by weight or 57.06 per cent by volume of alcohol and a sp. gr. of 0.9197 at 60°F. Spirits weaker or stronger than proof spirit are known as *Under* or *Over Proof* (U.P. or O.P.). A 20 per cent U.P. spirit means that 100 volumes of this spirit contain only 80 volumes of proof spirit, while a 20 O.P. spirit means that 100 volumes of this spirit when diluted with water would give 120 volumes of proof

spirit. Thus, the rectified spirit is 57.79 O.P. and absolute alcohol 75.35 O.P. spirit.

Methylated Spirit or Denatured Spirit.

Rectified spirit is made unfit for drinking purpose, or *denatured* as it is called, by mixing it with offensive smelling and unpalatable substances and is sold duty free for domestic use. Thus rectified spirit denatured by 0.5 per cent each of *pyridine bases* (obtained from coal tar and not from bone oil) and *caoutchoucine* (obtained by distilling scrap rubber) is sold in this country as *methylated spirit*. For industrial use, the rectified spirit is denatured by mixing it with various denaturants according to the nature of the industry. The term methylated spirit is a misnomer in this country, but it is in vogue on account of the fact that this spirit in European countries contains about 5-10 per cent of crude methyl alcohol or wood naphtha.

N.B. Real methylated spirit, i.e., spirit denatured with wood naphtha, may cause blindness if taken internally for some time but the so-called methylated spirit of this country would produce no such harmful effect on the eye under the same conditions.

Alcoholic Beverages.

The various alcoholic beverages are obtained either by directly fermenting different fruit juices which contain sugars or by fermenting the saccharine liquid obtained by the action of malt upon starchy grains. The fermented liquids are either directly consumed (like *wine, ale, beer*, etc.) or they are subsequently distilled to obtain *spirits* (such as *whisky, brandy, gin*, etc.) in which the percentage of alcohol is high.

A. Beverages from saccharine juices which are used without subsequent distillation:

(1) From grapes: champagne, 11-12% alcohol; port, 20-24%; sherry, 16-18%; etc.

(2) From apples: cider, 4-6%.

(3) From palm juice (palmyra palm and date palm): toddy or *Tari*, 4-6%.

B. Beverages from starchy grains, without subsequent distillation:

(1) From barley: beer 3-6%; ale 2-6%; etc.

(2) From rice: *saké*, 10-15%; *pachwai*, 6-12%; etc.

C. Beverages from saccharine juices, with subsequent distillation:

(1) From wine: brandy, 43-60%; etc.

(2) From molasses: rum, 43-60% ; Indian country liquor, 15-40%.

(3) From Mahua flowers: Indian country liquor, 15-40%.

D. Beverages from starchy grains, with subsequent distillation:

(1) From barley, wheat, maize, etc.: whisky, 43-60% ; gin, 38-50% ; etc.

Uses of Ethyl Alcohol.

It is a good *solvent* for many organic substances and is widely used in the laboratory for the isolation of drugs or for the purification of drugs and chemicals as also in various industries. Alcoholic solutions of drugs are known as *tinctures* and alcohol is, therefore, used in *pharmacy* for preparing tinctures and extracts since alcohol acts as a good preservative. It serves as the *starting material for medicinal chemicals* like ether, chloroform, chloral hydrate, iodoform, etc. Used as a *fuel* in motor cars it is known as "power alcohol." As *methylated spirit* it is used as *domestic fuel* for spirit stoves, etc., as well as for the preparation of *varnishes*, etc. Alcohol absorbed in soap gives *solidified spirit* which serves as a portable solid fuel for lamps, etc. Alcohol is used to *preserve anatomical and pathological specimens* and as an *antiseptic* and *sterilizing* agent in surgical practice.

Properties and Reactions of Ethyl Alcohol.

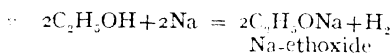
This is a mobile colourless liquid with a characteristic pleasant smell and a burning taste. The boiling point of anhydrous alcohol is 78.30° (760 mm.). As it freezes at a very low temperature (-112°) it is used in the preparation of minimum thermometers. Anhydrous alcohol has a sp. gr. of 0.7936 at 15° and 0.7894 at 20° . Pure alcohol is very hygroscopic and absorbs moisture when exposed to the air. It is miscible with water in all proportions. When mixed with water there is an evolution of heat and contraction of volume; a mixture of 52 vols. of alcohol and 48 vols. of water becomes 96.3 vols. Taken *internally*, alcohol diminishes oxidation in the body and shows an antipyretic action. It also favours the accumulation of fat in the body.

It is inflammable and burns in the air with a pale blue non-luminous flame, being oxidized to CO_2 and H_2O :

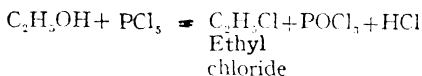
$C_2H_5OH + 3O_2 = 2CO_2 + 3H_2O$. Being a primary alcohol it is first oxidized to an aldehyde and then to an acid:



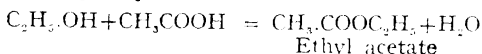
The oxidation to acetaldehyde can be effected by potassium dichromate and moderately strong sulphuric acid; if the reaction is allowed to continue acetic acid is produced. With metallic sodium, alcohol gives sodium ethoxide or alcoholate with the evolution of hydrogen:



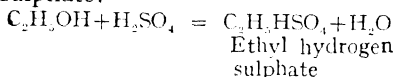
With phosphorus pentachloride, the OH group is replaced by chlorine:



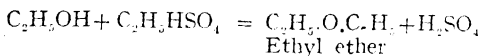
When heated with acetic acid in presence of a catalyst such as conc. H_2SO_4 ethyl acetate is obtained:



With concentrated sulphuric acid, alcohol forms ethyl hydrogen sulphate:



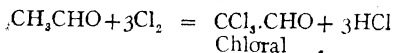
If the sulphuric acid is in excess, and the mixture is heated, ethylene is formed: $C_2H_5HSO_4 = CH_2:CH_2 + H_2SO_4$. If, on the other hand, the alcohol is present in excess, ethyl ether (p. 126) is formed:



Chlorine oxidizes alcohol to acetaldehyde:

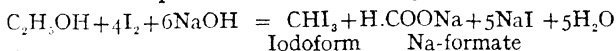


If there is excess of chlorine, chloral is formed:



Bromine produces a similar reaction.

Iodine, in presence of an alkali, gives iodoform (p. 69):



Like methyl alcohol ethyl alcohol also combines with certain inorganic salts forming 'alcohol of crystallization' similar to water of crystallization, *e.g.*, $\text{CaCl}_2 \cdot 4\text{C}_2\text{H}_5\text{OH}$, $\text{MgCl}_2 \cdot 6\text{C}_2\text{H}_5\text{OH}$, etc.

On account of this property of alcohol it cannot be dehydrated by anhydrous CaCl_2 and lime CaO is used instead.

Tests for Ethyl Alcohol.

(1) *Iodoform Reaction.* The equation for the reaction has been given above. To carry out the test, take 2—3 c.c. of a dilute solution of alcohol in a test tube, add 4 or 5 drops or more of a strong solution of I in KI to impart a brown colour and then add a 10 per cent solution of sodium carbonate or caustic soda drop by drop until a faint yellow colour remains. Warm on a water bath (temperature not exceeding 70°C) when the yellow crystals of iodoform, recognized by its smell and its hexagonal form under the microscope, will separate (Fig. 45, p. 467). The reaction is very sensitive and can detect even one part of alcohol in 1000 parts of water, but it must be remembered that it is given by other substances such as *acetone*, *acetaldehyde*, *isopropyl alcohol*, *amyl alcohol*, *lactic acid*, *ethyl acetate*, *acetoacetic ester*, etc. It is however, not given by methyl alcohol.

If only a trace of alcohol is present, the crystals of iodoform will separate after long standing and are detected only under the microscope.

(2) *Ethyl Acetate Test.* Take a few drops of the alcohol in a test tube containing 0.5 g. of anhydrous sodium acetate and add a few drops of conc. H_2SO_4 ; warm gently and pour into a dilute solution of sodium carbonate. A fruity odour of ethyl acetate is perceived.

(c) *Acetaldehyde Test.* Take 5 c.c. of a conc. aqueous solution of potassium dichromate, add 1 c.c. of conc. H_2SO_4 , warm and add a few drops of the alcohol. The solution turns green and a pungent odour of acetaldehyde is perceived. The aldehyde can be distilled over to another test tube containing some cold water; on adding Schiff's reagent to the distillate, a pink colour is obtained.

For other tests see Toxicology (p. 467)

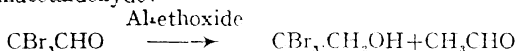
Determination of Alcohol in Wines, etc.

When mixed with water alone, the alcohol can be estimated by finding its specific gravity at 15.5°C (60°F), best with the help of a specific gravity flask. Tables are found in text books giving the percentage of alcohol both by volume and by weight and the corresponding sp. gr. at 15.5°C .

In *wines* or other alcoholic liquids, the alcohol is estimated as follows: A known volume (100 c.c.) of the liquid is neutralized with dilute caustic soda to fix the volatile acids present and transferred with a little water to a distillation flask connected with a vertical spiral condenser (Standard Revenue Still). About 80 c.c. of the distillate which contains the whole of the alcohol is collected in a 100 c.c. measuring flask and the volume made up to the mark with distilled water. The sp. gr. of the distillate is determined at 15.5°C. The percentage of the alcohol is then found out by referring to the tables. The distillation is necessary because the sugary substances and other extractives vitiate the sp. gr. and hence direct observation is useless.

Avertin, Tribromoethyl alcohol, $\text{CBr}_3\cdot\text{CH}_2\text{OH}$

This is prepared by the action of aluminium ethoxide on tribromacetaldehyde:



It is used as an anæsthetic and is given per rectum.

Some Higher Saturated Monohydric Alcohols.

n-Propyl Alcohol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$.—Occurs in fusel oil; colourless liquid, soluble in water, b.p. 97.19° (760 mm.), sp. gr. 0.8044 (20°).

Isopropyl Alcohol, $\text{CH}_3\text{CHOH}\cdot\text{CH}_3$. Prepared by reduction of acetone and used as a solvent; colourless liquid, miscible with water, b.p. 82.85° (760 mm.), sp. gr. 0.7887 (20°).

n-Butyl Alcohol, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$.—Prepared by the fermentation of starch and sugars with special bacteria (*Clostridium butylicum*); colourless liquid, dissolves in 11 volumes of water at 15°; used as solvent for lacquers and in the form of esters as plasticizers in cellulose paints and varnishes and the preparation of *cellophane*; b.p. 117° (760 mm.), sp. gr. 0.8097 (20°).

Isobutyl Alcohol, $(\text{CH}_3)_2\text{CH}\cdot\text{CH}_2\text{OH}$.—Occurs in fusel oil; colourless liquid; one part dissolves in 10 parts of water at 15°; b.p. 108° (760 mm.), sp. gr. 0.8003 (18°).

Tertiary Butyl Alcohol, $(\text{CH}_3)_3\text{C}\cdot\text{OH}$.—Occurs in fusel oil; colourless liquid, miscible with water, b.p. 82.55° (760 mm.), sp. gr. 0.7864 (20°).

n-Amyl Alcohol, $\text{CH}_3(\text{CH}_2)_4\text{CH}_2\text{OH}$.—Colourless liquid, insoluble in water; b.p. 137° (740 mm.); sp. gr. 0.8168 (20°).

Active Amyl Alcohol, $\text{C}_2\text{H}_5\cdot\text{CH}(\text{CH}_3)\cdot\text{CH}_2\text{OH}$. The laevorotatory alcohol occurs in fusel oil; $[\alpha]_D^{20} = -5.9^\circ$; colourless liquid, b.p. 128°, sp. gr. 0.816 (20°).

Isoamyl Alcohol, Fermentation Amyl Alcohol, $(\text{CH}_3)_2\text{CH}\cdot\text{CH}_2\text{CH}_2\text{OH}$.—Chief constituent of fusel oil; colourless liquid; 100 c.c. water dissolve 3.28 c.c. alcohol, b.p. 130° (760 mm.), sp. gr. 0.8064 (25°).

Tertiary Amyl Alcohol, Amylene Hydrate, $(\text{CH}_3)_2\text{C}(\text{OH})\text{C}_2\text{H}_5$.—Prepared by treating trimethyl ethylene (amylene), $(\text{CH}_3)_2\text{CH}:\text{CH}.\text{CH}_3$, with sulphuric acid and distilling the amylene sulphate formed with an alkali; colourless liquid with a sharp smell like that of camphor and peppermint and a pungent taste; dissolves in 8 parts of water; m.p.— 12° , b.p. 102° ; sp. gr. 0.8143 (15°); used as a hypnotic.

n-Octyl Alcohol, $\text{CH}_3(\text{CH}_2)_6.\text{CH}_2\text{OH}$.—Occurs as an ester of acetic acid in the essential oil from the common cow parsnip, *Heracleum sphondylium* L.; liquid with aromatic smell, m.p.— 15° , b.p. 195.5° , sp. gr., 0.8266 (21°).

Cetyl Alcohol, n-Hexadecyl Alcohol, $\text{CH}_3(\text{CH}_2)_{14}.\text{CH}_2\text{OH}$.—Occurs in *spermaceti*, the wax found in the head of sperm whales, as an ester of palmitic acid; colourless solid, soluble in ether and petroleum ether and in hot alcohol, insoluble in water; crystallizes in plates; m.p. 48° sp. gr., 0.8105 (60°).

Ceryl Alcohol, $\text{C}_{26}\text{H}_{53}\text{OH}$.—Occurs in Chinese wax as the ester of cerotic acid; colourless solid, insoluble in water; m.p. 79° .

Myricyl Alcohol, Melissyl Alcohol, $\text{C}_{30}\text{H}_{61}\text{OH}$.—Occurs in bees wax as an ester of palmitic acid; colourless solid, insoluble in water; crystallizes in plates; soluble in benzene, petroleum ether and hot alcohol, m.p. 88° .

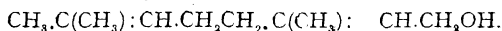
B. Unsaturated Monohydric Alcohols.

The unsaturated alcohols exhibit the chemical properties of alcohols and unsaturated hydrocarbons.

Allyl Alcohol, $\text{CH}_2 = \text{CH}.\text{CH}_2\text{OH}$

It occurs to a very small extent in wood spirit. It can be prepared by heating glycerol with oxalic acid and a little ammonium chloride to about 220° — 230° . It is a colourless liquid with an irritating smell; miscible with water in all proportions; b.p. 96.6° (760 mm.); sp. gr. 0.8540 (20°). The monovalent unsaturated group C_3H_5 , or $\text{CH}_2:\text{CH}.\text{CH}_2$ —is known as the *allyl radical*.

Geraniol, 2:6-Dimethyl octadiene-2:6-ol.



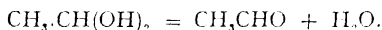
This is found in the free state in essential oils like citronella oil, oil of palmarosa, oil of rose geranium, etc. Colourless liquid with a smell of rose; b.p. 230° (760 m.m.), sp. gr. 0.8829 (15.5°).

CHAPTER IX

POLYHYDRIC ALCOHOLS AND ENZYMES

A. Dihydric Alcohols or Glycols.

We have already seen that by the substitution of one atom of hydrogen in ethane $\text{CH}_3.\text{CH}_3$ by a hydroxyl group we get the monohydric alcohol $\text{CH}_3.\text{CH}_2\text{OH}$. It may, therefore, be expected that a dihydric alcohol, ethylene glycol $\text{CH}_2\text{OH}.\text{CH}_2\text{OH}$, would result by the substitution of two atoms of hydrogen attached to two different carbon atoms of ethane by two hydroxyl groups. As compounds having two OH groups attached to the same carbon atom are very unstable (an exception being chloral hydrate, see p. 118) the substitution of two atoms of hydrogen attached to the same carbon atom of ethane by two hydroxyl groups would result in the formation of the unstable compound $\text{CH}_3.\text{CH}(\text{OH})_2$ which readily loses a molecule of water and gives an aldehyde:



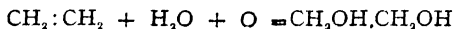
Dihydric alcohols may be expected from other hydrocarbons and we know of such compounds as propylene glycol $\text{CH}_3.\text{CH}(\text{OH}).\text{CH}_2\text{OH}$, trimethylene glycol $\text{CH}_2\text{OH}.\text{CH}_2.\text{CH}_2\text{OH}$, and so on. All these dihydric alcohols are designated as *glycols*, the word being derived from the simplest member ethylene glycol which is also known simply as glycol from its sweet taste (Gk. *glukus*—sweet)

Ethylene Glycol, Glycol, CH_2OH

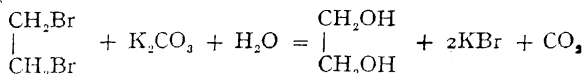


Synthesis.

(1) By the action of dilute potassium permanganate on ethylene:

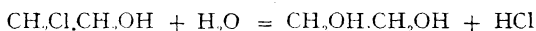
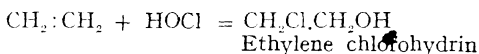


(2) By boiling ethylene dibromide with a dilute solution of potassium carbonate:



Preparation.

On a large scale, this is prepared by treating ethylene with hypochlorous acid at a low temperature to obtain ethylene chlorohydrin. The latter is then boiled with a mild alkali like sodium bicarbonate or milk of lime and glycol is formed. The solution is concentrated and the glycol extracted with a mixture of alcohol and ether.

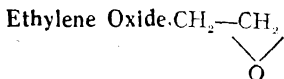
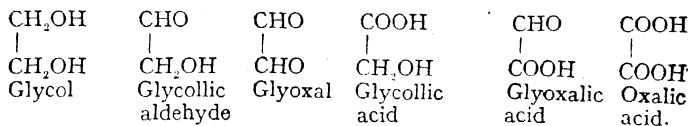


Properties, Uses and Reactions.

It is a thick, colourless liquid with a sweet taste, m.p. -13.6° , b.p. 197° (760 mm.), sp. gr. 1.1097 (25°). It is readily soluble in water and alcohol, but sparingly so in ether, 100 parts of ether dissolving only 1.1 parts of glycol. It is hygroscopic.

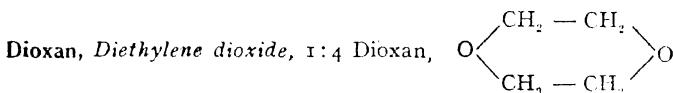
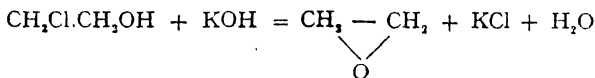
It is used as an antifreeze for aeroplane and motor car radiators, since a mixture of glycol and alcohol freezes at a very low temperature. It is also used as a solvent, as a preservative and as a substitute for glycerol.

As a dihydric alcohol, glycol gives all the reactions of the OH group with reagents like metallic sodium, phosphorus pentachloride, acetyl chloride, etc. On oxidation it may produce a variety of aldehydes and acids as will be apparent from the following formulæ:

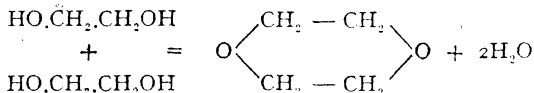


This is obtained by distilling ethylene chlorohydrin with a solution

of caustic potash. It is a gas, soluble in water, b.p. 12.5° ; used as an insecticide.



This is prepared by distilling glycol with concentrated H_2SO_4 or anhydrous ZnCl_2 :



This is a mobile liquid with an aromatic smell, soluble in alcohol and ether, and is used as a solvent in industries; b.p. 101° (760 mm.); sp. gr. 1.0329 (20°); m.p. 11° .

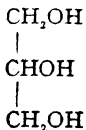
Triethanolamine, $\text{N}(\text{CH}_2.\text{CH}_2.\text{OH})_3$

This is prepared by passing ethylene oxide into an excess of a well-cooled concentrated ammonia and allowing the mixture to react for several hours. The mixture of mono-, di- and tri-ethanolamines thus formed is separated by fractional distillation in vacuo. The tri-derivative distils at 150 mm. between 250° — 280° . It is a hygroscopic liquid and its salts with oleic, palmitic or stearic acid are used as emulsifiers in the manufacture of many pharmaceutical preparations.

B. Trihydric Alcohols.

The trihydric alcohols are obtained by the substitution of three hydrogen atoms attached to three different carbon atoms of a hydrocarbon by OH groups. We may expect various trihydric alcohols, such as trihydroxy propane or glycerol $\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{OH}$, α , β , γ -trihydroxy butane $\text{CH}_3.\text{CHOH}.\text{CHOH}.\text{CH}_2\text{OH}$, α , β , γ -trihydroxy pentane $\text{CH}_3.\text{CH}_2.\text{CHOH}.\text{CHOH}.\text{CH}_2\text{OH}$, and so on. Of these, glycerol is the only member which is of much medicinal importance.

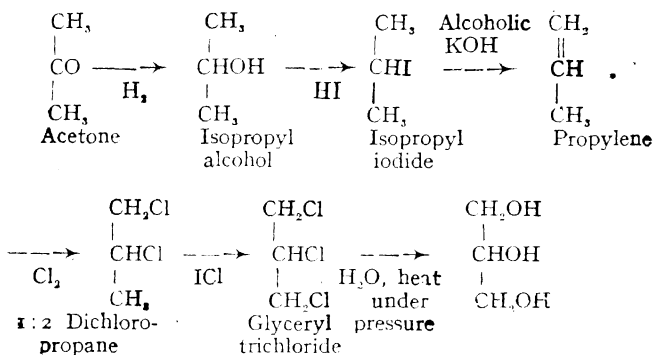
Glycerol, Glycerine, Trihydroxypropane, $\text{C}_3\text{H}_5(\text{OH})_3$,



Occurrence.—This is a normal constituent of all natural fats and oils which are the glyceryl esters of the higher fatty acids. It is formed in small quantities during the alcoholic fermentation of sugars and it is stated to be present in minute amounts in normal blood.

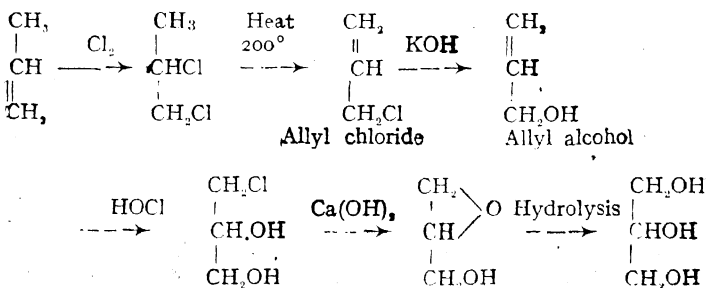
Synthesis of Glycerol.

(1) From acetone:



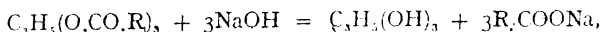
This synthesis clearly establishes the constitution of glycerol.

(2) From Petroleum: Propylene is readily obtained by the cracking of petroleum at a suitable high temperature and pressure, and from propylene glycerol can be synthesized as shown above or by the following method:

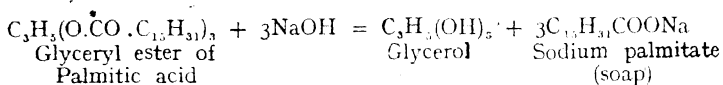


Manufacture of Glycerol.

(3) *As a By-product of Soap Manufacture.* When a neutral fat or oil is boiled with caustic soda, the glycerol is liberated according to the following equation and soap (*i.e.*, a mixture of the sodium salts of the higher fatty acids) is formed, *e.g.*,



where R is a higher alkyl radical. For instance,

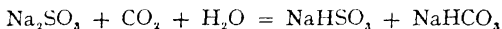


The oil or fat from animal or vegetable sources is taken in a large cylindrical iron vessel, known as the soap kettle, and heated in a current of steam with the calculated amount of NaOH. When the hydrolysis (saponification) is complete, some common salt is added in order to "salt out" or separate the soap which being less soluble in brine floats up. The lower aqueous layer, known as the *spent lye*, contains from 4 to 8 per cent of glycerol together with various impurities. It is first faintly acidified with HCl and aluminium sulphate added in order to precipitate all the dissolved soap and fatty acids and filtered. The filtrate is made slightly alkaline and concentrated in steam heated pan under reduced pressure. When the concentration of glycerol reaches about 80 per cent, it is decolourized by treatment with activated charcoal and filtered. The liquid is finally distilled in high vacuum when glycerol is obtained as a colourless liquid.

* (4) *As a By-product in the Manufacture of Candles.*—A mixture of paraffin wax and stearic acid and other solid fatty acids is used in the manufacture of candles. To obtain these higher fatty acids, fats are hydrolyzed by superheated steam with a little sulphuric acid or lime. The *spent lye* obtained in this process is utilized for the manufacture of glycerol exactly in the same way as described above.

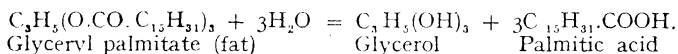
(5) *By the Fermentation of Sugars.*—It has been mentioned before that during the alcoholic fermentation of sugars by yeast a certain amount of glycerol (3 to 4 per cent)

is produced as a by-product. Due to a shortage of fats and oils this *biological process* has been improved during the Great War of 1914-1918 and an yield of 20 to 24 per cent of glycerol has been attained by adding some sodium sulphite at intervals during the process of fermentation, the sulphite being converted into bisulphite by the action of CO_2 :



The glycerol is recovered and purified as before by distillation under reduced pressure.

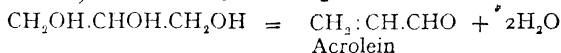
(4) Glycerol may also be prepared by the action of a fat-splitting enzyme, *lipase*, on fats and oils. This enzyme occurs in oil seeds, notably in castor seeds, and the hydrolysis proceeds as follows under suitable conditions:



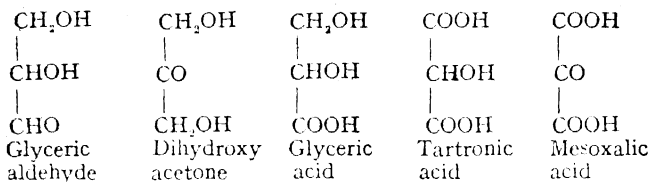
Properties and Reactions.

Glycerol is a colourless, odourless, syrupy liquid having a sp. gr. of 1.2604 at 20°. It has a sweet taste and is hygroscopic. It mixes with water and alcohol in all proportions but is insoluble in ether and chloroform. The presence of many OH groups accounts for its sweet taste, solubility in water and alcohol and its insolubility in ether. It is soluble in ethyl acetate to the extent of about 9 per cent. It boils at 290° with slight decomposition but it can be distilled under reduced pressure without any change; b.p. 162° (10 mm.), 210° (50 mm.). It is volatile with steam. Glycerol dissolves many inorganic substances such as borax, boric acid, $\text{Ca}(\text{OH})_2$, lead oxide, alum, etc.

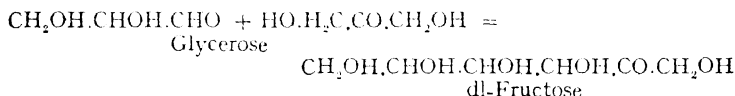
When heated with dehydrating agents like P_2O_5 or KHSO_4 , glycerol gives the unsaturated aldehyde, acrolein, which is recognized by its peculiar pungent odour:



On oxidation, glycerol gives rise to a variety of products which depend upon the nature of the oxidizing agent used. Thus we may get:

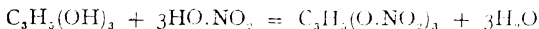


A mixture of glyceric aldehyde and dihydroxy acetone, obtained by the oxidation of glycerol with dilute nitric acid, is called *glycerose*. When glycerose is treated with an alkali it yields the optically inactive (dl) fructose by the process known as *aldol condensation* (see p. 116):



When HCl gas is passed through glycerol, monochlorohydrin of glycerol is formed $\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{Cl}$. But when a mixture of 1 part of glycerol and 1 part of glacial acetic acid is saturated with gaseous HCl and heated to 130° , the two terminal OH groups are replaced by Cl and dichlorohydrin of glycerol is formed, $\text{CH}_2\text{Cl}.\text{CHOH}.\text{CH}_2\text{Cl}$, which may be reduced to secondary propyl alcohol by nascent hydrogen. By the action of PCl_3 , all the OH groups are replaced by Cl and *glyceryl trichloride*, $\text{CH}_2\text{Cl}.\text{CHCl}.\text{CH}_2\text{Cl}$, is formed which resembles chloroform in smell.

When treated with a mixture of strong HNO_3 and H_2SO_4 , it forms nitroglycerine or glyceryl trinitrate:



It is readily oxidized by KMnO_4 with evolution of so much heat that it catches fire and burns with mild explosion. The glycerol is oxidized to CO_2 and H_2O , and the KMnO_4 is reduced to MnO_2 and KOH , and the residue becomes strongly alkaline.

Uses of Glycerol.

Glycerol is used in medicine for soothing inflamed surfaces as in the preparation of throat paints, etc., for the preparation of glycerophosphates, for the manufacture of cosmetics, transparent soaps, *plastics* (*glyptal resins*) and

in colour printing, as an antifreeze in motor car radiators in cold countries, as a preservative for fruits and to a large extent in the preparation of the explosive nitroglycerine, which plays a very important part in modern warfare.

Tests for Glycerol.

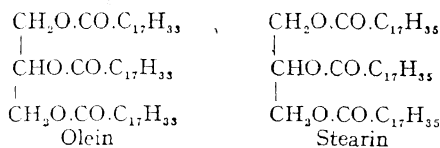
(1) When heated with potassium bisulphate KHSO_4 it yields vapours of acrolein which is recognized by its characteristic pungent odour as also by its blackening a filter paper moistened with a solution of ammoniacal silver nitrate.

(2) Take 2 or 3 c.c. of a 5 per cent solution of borax and add 2 drops of phenolphthalein, a red colour is obtained; add a neutral solution of glycerol in water (about 20 per cent) drop by drop, the red colour disappears. This disappearance of colour is due to the formation of sodium glyceroborate which is less alkaline than borax. Warm and the red colour appears; cool and the colour disappears (*Dunstan's Test*). Other polyhydric alcohols give this test but not so strongly as glycerol.

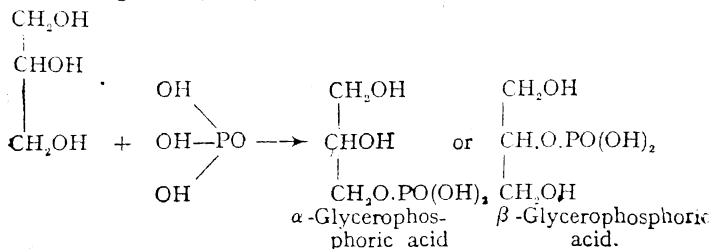
(3) Heat borax and Na_2CO_3 in a platinum loop and moisten with glycerol a green flame is produced.

Derivatives of Glycerol.

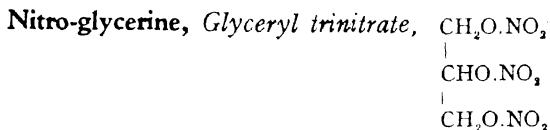
Glycerides and Glycerophosphates—The esters of glycerol with organic acids are known as *glycerides*. The *fats* and *oils* (see p. 165) are all glycerides of higher fatty acids. Thus *stearin* or *glyceryl tristearate*, found in fair amounts in mutton fat, is the ester of glycerol with three molecules of stearic acid. Similarly *olein* is glyceryl trioleate, and so on.



On heating glycerol with phosphoric acid, we get either α - or β -glycerophosphoric acid:



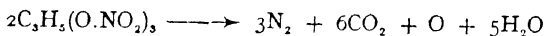
Lecithin, a lipid (p. 173) found in animal and vegetable tissues, is a derivative of α -glycerophosphoric acid. The *calcium* and *iron* salts and various other preparations of glycerophosphoric acid are used in medicine.



This is prepared by treating glycerol with a well-cooled mixture of fuming nitric acid and concentrated sulphuric acid. The ester is then washed with water and dilute sodium carbonate.

This is a colourless, odourless, oily liquid with an initial sweet but a burning after taste. It has a sp. gr. of 1.6009 at 15° and a m.p. of 13.2°. One gram dissolves in about 800 c.c. of water or in about 4 c.c. of absolute alcohol; it is miscible with ether, chloroform and glacial acetic acid. It is poisonous and its vapour produces headache and even loss of consciousness.

It is called Nobel's Explosive Oil since it explodes with terrific violence when it is struck or heated quickly in bulk. This explosion is due to the sudden liberation of a large volume of gas by its decomposition:



If, however, it is ignited in small quantities at a time it burns quietly.

Dynamite is made by mixing nitroglycerine with *Kieselguhr*, a very porous and light diatomaceous earth consisting mainly of silica. Many other explosives such as blasting gelatine (gelignite) for mining, and *cordite* or *smokeless powder* for rifle cartridges, etc., are made by mixing nitroglycerine with various substances. It was Alfred Nobel, a Swedish chemist, who first (1867) prepared safe explosives with mixtures of nitroglycerine and other substances. The Nobel Prize is given in his name.

Nitroglycerine is used in medicine in 1 per cent alcoholic solution (Liquor Trinitrini) in very small doses in asthma,

in lowering high blood pressure, and in heart troubles (angina pectoris). The name nitro-glycerine is misleading as it is not a *nitro* compound but an *ester* of nitric acid. When treated with KOH it is hydrolyzed to glycerol and KNO_3 , whereas a nitro compound (e.g. $\text{C}_2\text{H}_5\text{NO}_2$) is not hydrolyzed under the same conditions.

C. Some higher Polyhydric Alcohols.

Tetrahydric Alcohols. $\text{CH}_2\text{OH}(\text{CHOH})_2\text{CH}_2\text{OH}$; e.g. *Erythritol*, Erythrol or Erythrite ; found as an ester in lichens and in some sea weeds ; crystalline solid with a sweet taste, m.p. 89° (d-) ; soluble in water and in boiling alcohol but insoluble in ether. Its tetranitrate is an explosive and also used in medicine.

Pentahydric Alcohols. $\text{CH}_2\text{OH}(\text{CHOH})_3\text{CH}_2\text{OH}$; e.g. *Arabitol* ; prepared by reducing arabinose ; crystalline solid with a sweet taste ; easily soluble in water and in boiling alcohol ; m.p. 102° (l-). Another pentahydric alcohol *adonitol* or adonite is used in making bacteriological media.

Hexahydric Alcohols, $\text{CH}_2\text{OH}(\text{CHOH})_4\text{CH}_2\text{OH}$; e.g.,

Mannitol, *Mannite* ; occurs in *manna*, the dried sap of various plants ; crystalline solid ; m.p. 166° ; 100 parts of water dissolve 18.5 parts of d-mannite at 23° ; slightly sweetish in taste.

Dulcitol, *Dulcite* ; found in the manna of some Madagascar plants ; crystalline solid, m.p. 188.5° ; sweetish in taste ; 100 parts of water dissolve 2.94 parts at 16.5° .

Sorbitol or sorbite is another hexahydric alcohol.

All these polyhydric alcohols are used in bacteriology for preparation of media for fermentation tests.

ENZYMES

In a previous chapter we have seen that living cells like yeast, bacteria and moulds secrete various *enzymes* or *organic catalysts* known as *ferments*. Like inorganic catalysts (*Kata*—down, *lysis*—loosening), these enzymes increase the rate of a reaction which would ordinarily proceed very slowly. And since bacteria, yeasts and moulds multiply at an enormously rapid rate when they get suitable media they are now increasingly utilized in the so-called Biological Processes for the manufacture of many chemicals produced by the action of enzymes, and they are more effective than many inorganic catalysts which need more drastic treatment like pressure, heat, acids or alkalies.

Büchner in 1897 showed that a juice freed from living cells, obtained by crushing yeast cells with sand and filtering under pressure, was capable of fermenting sugars like the living yeast. Similar other proofs have demonstrated that although the enzymes are produced by living cells, life is not essential for the activity of these enzymes.

Occurrence and Distribution.—These enzymes are found not only in yeasts, moulds or bacteria but also in all living tissues, vegetable or animal. They are, therefore, widely distributed in nature. They are present, however, in very minute amounts and several types of enzymes may be present together. It is thus very difficult to obtain them in a pure condition.

Isolation and Purification of Enzymes. The method varies a good deal with the nature of the material, but the general principles of isolation and purification may be briefly stated as follows: The fresh, finely minced material is thoroughly ground up with about twice the bulk of water, containing some mild antiseptic like chloroform or toluene, and the extract is filtered. The filtrate may be dialyzed to remove all crystalloids and it is treated with 3 to 4 times its volume of alcohol. The precipitated enzyme is filtered, washed with alcohol and then with ether and finally dried at room temperature in a vacuum over conc. H_2SO_4 . The preparation is likely to contain proteins and other impurities. For further purification, the material is redissolved in a small bulk of water and the enzyme adsorbed by substances like kaolin, alumina, etc., at a suitable pH and subsequently liberated from the adsorbing material at another pH by the use of dilute acids or alkalis and precipitated again with alcohol, washed and dried as before.

Chemical Nature of Enzymes.—The exact chemical nature of many of them has not as yet been established. They are all colloidal in nature and some of them like urease, pepsin and trypsin have been prepared in a crystalline state. Chemical studies of some of the best purified enzymes show that they are *protein* in nature, giving the typical colour reactions of proteins and yielding amino acids on hydrolysis. The catalytic activity of the crystalline preparations of urease, pepsin and trypsin has been shown to depend upon the protein molecule remaining intact and any change in the original molecule affects their activity. Again, malt amylase has been shown to possess a definite iso-electric point which

is characteristic of all native proteins. These and other evidences support the view that many of the enzymes are protein in nature.

Mode of Action of Enzymes.—As regards the mechanism of enzyme action it is believed that the enzyme, which is colloidal in nature, first adsorbs the *substrate* (the substance upon which an enzyme reacts). The substrate then breaks down into some decomposition or hydrolytic products leaving a loose complex of another product with the enzyme. This latter complex slowly breaks down and makes the enzyme inactive. This theory explains the gradual disappearance of the enzyme during the reaction.

Nomenclature of Enzymes.—An enzyme is generally designated by the suffix—*ase* added to the name of the substance upon which it acts (substrate). Thus, *maltase* is an enzyme which hydrolyzes maltose, *amylase* (Lat. *amylum*—starch) hydrolyzes starch, *lipase* (Gk. *lipos*—fat) hydrolyzes fats, *protease* hydrolyzes proteins, and so on. Certain well-established old names such as pepsin, trypsin, etc., have however been left unchanged. Many of the enzymes possess the property of hydrolyzing their substrates and they are, therefore, called *hydrolytic* enzymes. Others produce changes known as fermentation, others again act as oxidizing agents and are known as *oxidases*, and so on. Some enzymes require the presence of another substance for its activity and this is known as a *coenzyme*. A simple classification of the more common types of enzymes is given below.

Classification of Enzymes:

A. Hydrolytic Enzymes.

I. CARBOHYDRATE SPLITTING ENZYMES (Sacroclastic).

Diastase (Amylase): occurs in germinating grains, in malt, etc.; hydrolyzes starch to dextrin and maltose; the amylase secreted by salivary glands, known as *ptyalin*, or that secreted by pancreas, known as pancreatic amylase or *amyllopsin*, is also known to convert starch into maltose.

Maltase (α -glucosidase): occurs in germinating grains, malt, yeast, intestinal mucosa, etc.; converts maltose into glucose. It also hydrolyzes α -glucosides but not β -glucosides.

Invertase (Sucrase): occurs in yeast, in intestinal mucosa, etc.; converts cane sugar into glucose and fructose.

Lactase: occurs in intestinal mucosa; converts lactose into glucose and galactose.

2. GLUCOSIDE SPLITTING ENZYMES.

Emulsin (β -glucosidase): occurs in bitter almonds; hydrolyzes β -glucosides but not α -glucosides; hydrolyzes amygdalin (a cyanogenetic β -glucoside) into glucose, HCN and benzaldehyde.

α -Glucosidase (Maltase): mentioned above.

Myrosin: occurs in mustard seeds; hydrolyzes the glucoside sinigrin found in mustard into glucose, allylisothiocyanate and KHSO_4 .

Linase: occurs in linseed; hydrolyzes the cyanogenetic glucoside linamarin present in linseed into glucose, HCN and acetone.

3. ESTER OR FAT SPLITTING ENZYMES (Lipolytic).

Lipase: occurs in castor seeds, in gastric mucosa, pancreas, etc.; hydrolyzes fats into glycerol and fatty acids; hydrolyzes other esters into their components.

Esterase: found in livers of animals; hydrolyzes esters into the corresponding alcohol and acid.

PROTEIN SPLITTING ENZYMES (Proteoclastic).

Pepsin: found in gastric mucosa; hydrolyzes proteins to proteoses and peptones.

Trypsin: probably a mixture of enzymes; found in pancreas; hydrolyzes proteins to proteoses, peptones, polypeptides and amino acids.

Papain: found in the milky juice obtained from the leaves, fruits, stems and roots of the papaya tree (*Carica papaya* Linn.); hydrolyzes proteins to peptones and amino acids.

Bromelin: found in the juice of ripe pineapples; hydrolyzes proteins to peptones and amino acids.

B. Fermenting Enzymes.

e.g., Zymase: occurs in yeast; ferments glucose producing alcohol, carbon dioxide, etc.; ferments other hexoses like fructose, mannose and galactose but does not ferment lactose and pentoses.

Lactic Acid Ferment: produced by *Bacillus acidi lactici* as well as by other bacteria; decomposes lactose, cane sugar or glucose into lactic acid and other products.

Butyric Acid Ferment: produced by butyric acid bacteria known as *Clostridium butyricum*; ferments glucose into butyric acid, acetic acid, etc.

Butyl Alcohol Ferment: produced by butyl alcohol bacteria known as *Clostridium butylicum*; ferments glucose into butyl alcohol, acetone, etc.

C. Coagulating Enzymes.

e.g., Rennin: found in the mucous coat of the stomach of pigs and calves; coagulates milk.

Thrombin: present in blood plasma; clots blood.

D. Oxidizing Enzymes (Oxidases).

e.g., Catalase: found in animal and vegetable tissues; decomposes a solution of hydrogen peroxide to water liberating molecular oxygen.

Uricolase: present in liver; decomposes uric acid to allantoin.

Peroxidase: found in vegetable tissues; decomposes a solution of hydrogen peroxide or an organic peroxide and liberates nascent oxygen; the nascent oxygen can be recognized by its action on some easily oxidizable substance like tincture of guaiacum, benzidine, etc.

β -Oxybutyrase: found in liver; oxidizes β -hydroxybutyric acid to aceto-acetic acid.

Dopa oxidase: occurs in human skin; oxidizes derivatives of tyrosine to melanin, a skin pigment.

E. Deaminases.

e.g., Urease: found in soya beans, in the beans of *Canavalia ensiformis* DC. ("makhan sheem") and in other

beans, in arhar dāl (*Cajanus indicus*) and in some bacteria and fungi; decomposes urea into ammonia and carbon dioxide (see urea, p. 252).

Guanase: found in spleen, liver, etc.; converts the purine guanine into xanthine.

Arginase: found in liver and kidney; converts arginine into ornithine and urea.

F. Carboxylases.

e.g., *Carboxylase*: found in yeast and in animal tissues; converts amino acids into carbon dioxide and amines.

G. *Reductases*: found in yeast; converts acetaldehyde into ethyl alcohol.

Some Properties of Enzymes.

As mentioned before, the enzymes are *colloidal* in nature and can thus adsorb other substances. They are *soluble in water*, dilute glycerol and dilute salt solutions but are precipitated by alcohol or by saturation with ammonium sulphate.

Like inorganic catalysts these enzymes *increase the rate of a reaction* which proceeds normally at a very slow rate, and they can *transform many times their weight of the substrate*. The reaction proceeds faster with the concentration of the enzymes but unlike inorganic catalysts these enzymes *gradually disappear during the reaction*.

Unlike inorganic catalysts the action of the enzymes is more or less *specific in nature*. Thus a fat splitting enzyme will never hydrolyze a carbohydrate, and a proteolytic enzyme will never hydrolyze a fat. This specificity is very marked in some cases. Thus invertase will act upon cane sugar but not upon milk sugar, and maltase will act upon α -glucosides but not upon β -glucosides. This specificity is probably connected with the structure of the substrate as well as that of the enzyme. Fischer has suggested that the relation between the substrate and the enzyme is similar to that between a *lock and its key*, viz., the enzyme (key) will not act upon the substrate (lock) unless the structures fit each other.

Unlike inorganic catalysts, the enzymes *act within a narrow range of temperature*. They are all destroyed by heating to about 80°C in the presence of water and to 100°C when dry. In the majority of cases we cannot heat them with safety above 60°C . At low temperatures again the action of an enzyme is *very slow*. The temperature which is most favourable for its activity is known as the *optimal temperature* and it varies with the nature of the enzyme. Generally, however, most of the enzymes especially those of animal origin act best between 35° and 45°C and their optimum temperature is believed to be about 37°C .

Another factor which influences the activity of an enzyme is the *reaction of the medium* and each enzyme probably acts best within a narrow range of pH. Thus pepsin acts best in acid medium (pH 1.4) and trypsin acts best in alkaline medium (pH 8.3) while papain acts both in acid and alkaline media. Neutral or slightly alkaline medium is, however, suitable for most of the enzymes.

Some enzymes require certain *salts* for their activity. For example, zymase requires phosphates, diastase requires chlorides, oxidizing enzymes require iron or manganese salts, lipase requires bile salts, and so on.

Catalytic poisons such as HCN, H.CHO , etc., react just in the same way with the enzymes as they do with the inorganic catalysts.

The *end products* formed in a reaction also influence the activity of an enzyme. Thus we have seen that 14 per cent alcohol almost stops the action of zymase.

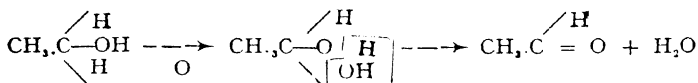
The action of an enzyme is sometimes *reversible*. Thus lipase which hydrolyzes ethyl butyrate is also capable of synthesizing the same from ethyl alcohol and butyric acid.

CHAPTER X

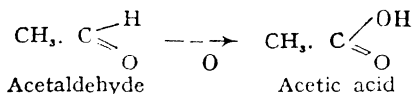
ALDEHYDES AND KETONES

A. Saturated Aldehydes.

If a primary alcohol such as ethyl alcohol $\text{CH}_3\text{CH}_2\text{OH}$ is carefully oxidized with a mild oxidizing agent like potassium dichromate and dilute sulphuric acid, it is changed into an aldehyde known as acetaldehyde, CH_3CHO . The word aldehyde is derived from *al* (cohol) *dehyd* (rogenatus) since it is obtained from an alcohol and contains two atoms of hydrogen less than the corresponding alcohol. The mild oxidation of a primary alcohol takes places as follows:



Since two hydroxyl groups cannot remain attached to the same carbon atom, the intermediate stage shown above cannot exist as such and breaks up at once into acetaldehyde with the elimination of a molecule of water. On further oxidation an aldehyde yields an acid containing the same number of carbon atoms:

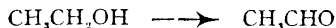


Nomenclature of Aldehydes.

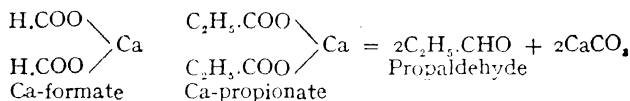
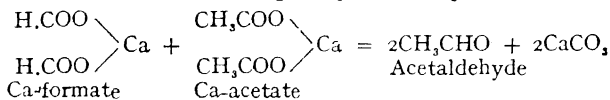
The name of an aldehyde is derived from the corresponding acid formed by its oxidation. Thus, the aldehyde which yields formic acid is termed formaldehyde, the aldehyde that yields acetic acid is called acetaldehyde, similarly propaldehyde, butaldehyde, and so on. In the international system of nomenclature the suffix *-al* is attached to the parent hydrocarbon after dropping the ending *-e*. Thus we have methanal, $\text{H}\cdot\text{CHO}$; from methane, ethanal, CH_3CHO , from ethane, propanal, $\text{CH}_3\text{CH}_2\text{CHO}$, from propane, and so on.

General Methods of Formation.

(1) Mild oxidation of a primary alcohol with potassium dichromate and dilute sulphuric acid:



(2) Dry distillation of calcium formate with the calcium salt of the corresponding fatty acid: *e.g.*,

**General Properties of Aldehydes.**

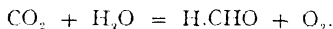
The aldehydes possess the characteristic group $\text{C} \begin{array}{l} \diagup \text{H} \\ \parallel \\ \text{O} \end{array}$

and have the general formula $\text{R}.\text{CHO}$, where R is H or any monovalent radical. The aliphatic aldehydes form the following *homologous series*: formaldehyde $\text{H}.\text{CHO}$ (b.p. -21°), acetaldehyde CH_3CHO (b.p. 20.8°), propaldehyde $\text{CH}_3\text{CH}_2\text{CHO}$ (b.p. 49.5°), n-butaldehyde $\text{CH}_3(\text{CH}_2)_2\text{CHO}$ (b.p. 75°), n-valeraldehyde $\text{CH}_3(\text{CH}_2)_3\text{CHO}$ (b.p. 102°), and so on. The lowest member, formaldehyde, is a gas and the next higher ones are colourless liquids. They are reduced to primary alcohols and oxidized to acids with the same number of carbon atoms. The aldehydes are very reactive bodies and combine with many substances to form additive and other compounds. They would even combine amongst themselves to form polymers and other products of condensation (see acetaldehyde).

Formaldehyde, Methanal, CH_2O , $\text{H}.\text{CHO}$

Occurrence and Natural Formation.—It has been experimentally demonstrated that CO_2 and H_2C , in presence of catalysts such as colloidal ferric hydroxide, are converted into formaldehyde under the influence of sunlight. The formation

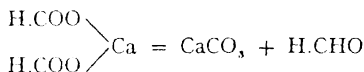
of sugars and other carbohydrates in plants from the CO_2 of the air in the presence of moisture under the influence of sunlight is known as *photosynthesis*. The green colouring matter of plants (chlorophyll) absorbs solar energy and transforms CO_2 and H_2O into formaldehyde and gives up O_2 :



The formaldehyde is at once polymerized into a sugar. The process is so rapid that it is difficult to detect the presence of formaldehyde formed during the photosynthesis. The sugar is either stored as such or in the form of other carbohydrates such as starch or cellulose.

Synthesis

(1) By heating calcium formate:



Preparation

(2) Formaldehyde is prepared *on a large scale* by passing a mixture of air and vapour of methyl alcohol through tubes containing copper gauze heated to about 600° or platinized asbestos heated to about 300° . The alcohol is oxidized by the oxygen of the air in presence of the metal Cu or Pt which acts as a catalyst. The vapour of formaldehyde is absorbed in water and a solution containing about 40 per cent of formaldehyde is obtained. The solution usually contains some methyl alcohol which escapes oxidation.

For a lecture demonstration, one may suspend a red hot platinum spiral (or foil) above the surface of some slightly warmed methyl alcohol kept in a beaker (Fig. 25). When properly adjusted, the platinum foil continues to glow indefinitely owing to the catalytic oxidation of the methyl alcohol at the surface of the spiral, and in a few seconds the smell of formaldehyde becomes perceptible and can be tested by exposing a filter paper soaked in Schiff's reagent.

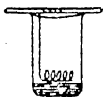
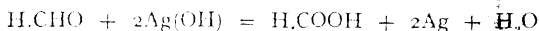


FIG. 25

Properties and Reactions of Formaldehyde.

Formaldehyde is a gas with a sharp pungent smell. Liquid formaldehyde boils at -21° . An aqueous solution containing about 40 per cent of formaldehyde is known as *formalin* or *formol*; it usually contains 8 to 15 per cent of methyl alcohol which prevents its polymerization.

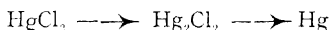
Formaldehyde is a strong reducing agent. It reduces an ammoniacal solution of silver nitrate to metallic silver which is deposited on the walls of the test tube as a mirror:



It reduces *Fehling's solution* (see glucose), a red precipitate of cuprous oxide being obtained:

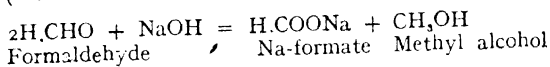


A solution of magenta decolorized by SO_2 , known as *Schiff's reagent* (see p. 80), regains its red colour on exposure to the vapour or by the addition of a solution of formaldehyde. Mercuric chloride is reduced to mercurous chloride and then to metallic mercury:

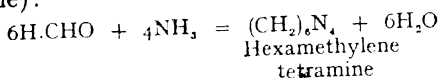


Polymerization: When a pure aqueous solution of formaldehyde is concentrated, the formaldehyde is converted into a solid amorphous substance known as *paraformaldehyde* or *paraform* $(\text{CH}_2\text{O})_n$. It has the same percentage composition as that of formaldehyde but its molecular weight is a multiple of the same; hence it is called a *polymer* and the transformation is known as *polymerization*. Paraform melts at 160° and it is soluble in water but insoluble in alcohol or ether. When heated it gives formaldehyde vapour and hence it is used as a disinfectant. When liquid formaldehyde is cooled below -20° it is gradually transformed into another polymer known as *metaformaldehyde* or *trioxymethylene* $(\text{CH}_2\text{O})_3$. This is a solid, m.p. 171° , and is insoluble in water. If a solution of formaldehyde is left with a weak alkali such as lime water or magnesium hydroxide, it is slowly polymerized into *formose* $\text{C}_6\text{H}_{12}\text{O}_6$, a mixture of sugars closely related to glucose. This reaction lends strong support to the view that formaldehyde forms an intermediate stage in the

photosynthesis of carbohydrates. With a strong aqueous solution of NaOH formaldehyde does not form a resin as in the case of acetaldehyde, but it gives a mixture of alcohol and acid (cf. aromatic aldehydes):

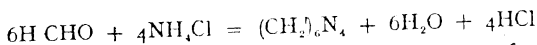


When treated with ammonia or its salts, formaldehyde gives a colourless crystalline solid known as *hexamethylene tetramine*, a compound having a cyclic structure (cf. acetaldehyde):



It is fairly soluble in water but less so in alcohol and chloroform and is almost insoluble in ether. It is used as an urinary antiseptic and is also known by other names such as *hexamine* (B.P.), *urotropine*, *cystamine*, etc.

The reaction of formaldehyde with ammonia or its salts can serve for the estimation of the latter, the free acid liberated in the latter case being titrated with a standard alkali:



Formaldehyde also reacts with the amino group of a neutral amino acid and forms a methylene compound (see p. 361).

Uses.—Formalin is used as a disinfectant and antiseptic. It is a good preservative, especially for anatomical specimens which are hardened without being opaque. Formaldehyde acts on casein, the protein found in milk, and forms a horn-like substance known as *galalith* which is used as artificial horn or ivory. Formaldehyde reacts with phenol and forms a hard resinous substance or plastic known as *bakelite* which is used in the manufacture of various household articles such as tea cup, ash tray, electric switch, etc. Formaldehyde is also used in tanning and in the manufacture of medicinal chemicals and of artificial silk.

Tests

- (1) Reduction of ammoniacal silver nitrate.

(2) Restoration of the pink colour of Schiff's reagent.

(3) Add 2 c.c. of a 1 per cent solution of phenylhydrazine hydrochloride (freshly prepared and filtered) to 10 c.c. of the liquid to be tested; then add 1 c.c. of a freshly prepared 5 per cent solution of potassium ferricyanide and 5 c.c. of conc. HCl—a brilliant pink or magenta colour is produced. The test is said to detect formaldehyde even in a dilution of 1 in a million (Schryver's test).

(4) To the solution of formaldehyde add 2 c.c. of a 1 per cent solution of phenylhydrazine hydrochloride and 2 c.c. of a freshly prepared solution of sodium nitroprusside; add an excess of dilute caustic soda, an intense blue colour is produced (Rimini's test).

(5) Marquis test (see methyl alcohol on p. 78 and morphine on p. 487).

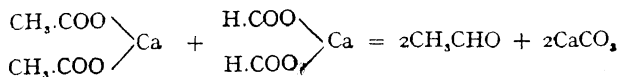
(6) Hehner's test—for the detection of formalin in milk; to 5 c.c. of milk suspected to contain formalin (used as a preservative but not permitted by law), add gently some conc. H_2SO_4 containing a trace of FeCl_3 ; a fine purple ring is formed at the junction.

Acetaldehyde, Ethanal, CH_3CHO .

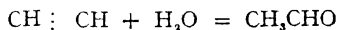
Occurrence.—It is found as a by-product in alcoholic fermentation.

Synthesis

(1) By heating calcium acetate with calcium formate:



(2) By passing acetylene into dilute sulphuric acid in presence of HgO or mercuric sulphate as a catalyst and then distilling it. This method is used on a commercial scale.



Preparation.

In the laboratory, acetaldehyde is prepared by oxidizing ethyl alcohol with a mixture of sodium dichromate and dilute sulphuric acid. (N.B. Sodium dichromate is preferred to potassium dichromate owing to its greater solubility).

A mixture of absolute alcohol (62 c.c.) and concentrated sulphuric acid (38 c.c.) is slowly dropped through a tap funnel into a solution of sodium dichromate (100 g) in water (200 c.c.) kept in a round bottomed flask warmed over a sand

bath (Fig. 26). The flask is connected through a delivery

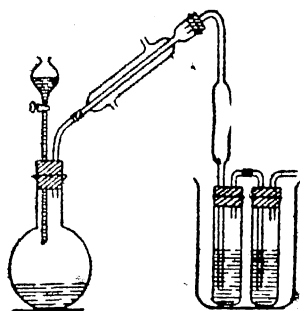
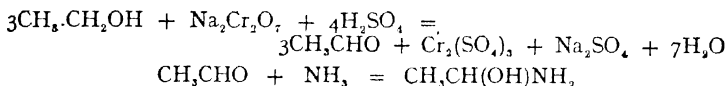


FIG. 26.

tube to an ascending condenser through which water (at about 30°) is circulated. The alcohol and water vapour are condensed back while the aldehyde which has a low boiling point passes over through the pipette connected with the condenser into the absorbing vessels. The aldehyde vapour is absorbed in dry ether kept in the cylinders which are kept cool with ice water in a large jar. The ethereal solution of the acetaldehyde is disconnected from the condenser and a current of dry ammonia gas passed until all the aldehyde is precipitated as colourless crystals of aldehyde ammonia. The crystals of aldehyde ammonia are taken out, dissolved in about the same weight of water and distilled from a small distilling flask with dilute sulphuric acid, the receiver being well cooled. The distillate is dried with anhydrous calcium chloride and redistilled. The reactions are as follows:—

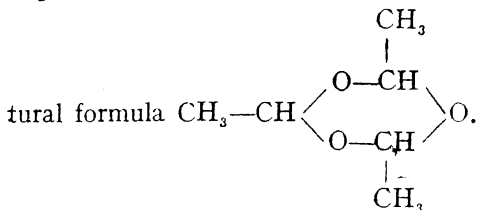


Properties and Reactions of Acetaldehyde.

Acetaldehyde is a colourless liquid with a characteristic smell but when inhaled the vapour produces a kind of cramp in the chest. It boils at 20.8° (760 mm.) and has a sp. gr. of 0.7876 at 16° . It is miscible with water, alcohol and ether and burns with a luminous flame.

It is a strong reducing agent, being itself oxidized to acetic acid. Thus it reduces, as in the case of formaldehyde, an ammoniacal solution of silver nitrate to metallic silver which is deposited as a mirror, and Fehling's solution on warming with a deposit of red cuprous oxide. It gives a pink colour with Schiff's reagent, a reaction common to all aldehydes.

Polymerization Reactions. When a little acetaldehyde is treated with a drop of conc. H_2SO_4 there is a rise of temperature and acetaldehyde is converted into *paraldehyde* $(\text{CH}_3\text{CHO})_3$, a liquid which boils at 124° and is insoluble in water. On cooling, it solidifies to a crystalline form, m.p. 12.6° . It is a polymer of acetaldehyde having the struc-



an example of the conversion of straight chain to a cyclic structure. It is used in medicine as a hypnotic. On distilling with dilute H_2SO_4 it is, however, reconverted into acetaldehyde.

When acetaldehyde is treated with dilute sulphuric acid or HCl at a low temperature (below 0°), it is converted into another polymer known as *metaldehyde* $(\text{CH}_3\text{CHO})_4$ which is a white crystalline solid insoluble in water but soluble in alcohol and ether. It sublimes without melting at 115° and it burns with a non-luminous flame. It is compressed into rectangular sticks and is used as a solid fuel, known as *meta fuel*, as a substitute for spirit. On distilling with dilute sulphuric acid metaldehyde yields acetaldehyde.

When an aqueous solution of acetaldehyde is warmed with a strong solution of caustic soda the liquid becomes dark and there is a deposit of a brown resinous mass known as *aldehyde resin* (cf. formaldehyde). If a very dilute solution of acetaldehyde is boiled with NaOH solution, a yellow or brown colouration is produced. This reaction serves as a test for acetaldehyde.

Addition Reactions. Acetaldehyde does not polymerize to form sugars as in formaldehyde. It forms many additive compounds. Thus with ammonia it gives a white crystalline

compound known as *aldehyde ammonia* $\text{CH}_3\text{CH} \begin{array}{c} \diagup \text{OH} \\ \diagdown \text{NH}_2 \end{array}$. With

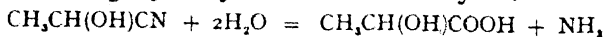
sodium bisulphite NaHSO_3 , it gives a white crystalline com-

pound known as *aldehyde bisulphite* $\text{CH}_3\text{CH} \begin{matrix} \text{OH} \\ \text{SO}_3\text{Na} \end{matrix}$

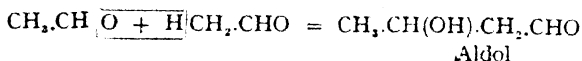
This reaction is used to separate an aldehyde from its mixture with other compounds, since the aldehyde can be regenerated by heating with acids or alkalies. With hydrocyanic acid, acetaldehyde gives *aldehyde cyanhydrin*

$\text{CH}_3\text{CH} \begin{matrix} \text{OH} \\ \text{CN} \end{matrix}$; this reaction is utilized in introducing a new

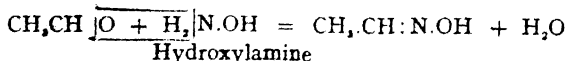
carbon atom into a molecule, and since the cyanogen group can be hydrolyzed to a COOH group it serves as a means of synthesizing hydroxy acids from aldehydes.



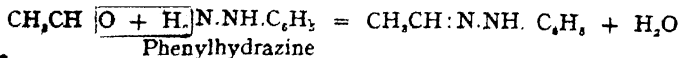
Condensation Reactions.—When acetaldehyde is treated with a solution of K_2CO_3 it is converted into *aldol*, the name being derived from the fact that the product is both an aldehyde and an alcohol:



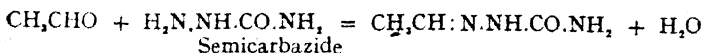
This reaction is known as *aldol condensation* and is common among aldehydes. It differs from polymerization in the fact that the latter is a reversible reaction. Strictly speaking, the word *condensation* is applied to the combination of two molecules of the same or different compounds with the elimination of a molecule of water. Thus, acetaldehyde condenses with hydroxylamine to form *acetaldoxime*:



Acetaldehyde condenses with phenylhydrazine to form *acetaldehyde phenylhydrazone*:



Acetaldehyde condenses with semicarbazide to give *acetaldehyde semicarbazone*:



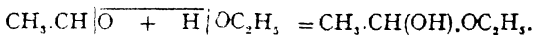
These oximes, phenylhydrazones and semicarbazides are crystalline compounds with characteristic melting points and serve to identify aldehydes and ketones (see).

Tests for Acetaldehyde

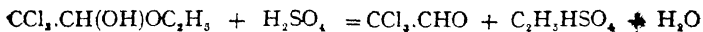
- (1) Reduction of ammoniacal silver nitrate.
- (2) Magenta colour with Schiff's reagent for all aldehydes (cf. formaldehyde).
- (3) A blue colour with sodium nitroprusside and piperidine.
- (4) A cherry red colour with sodium nitroprusside and caustic soda.
- (5) It gives the iodoform reaction (cf. alcohol).

Chloral, Trichloroacetaldehyde, CCl_3CHO

Chloral is obtained by the action of chlorine upon ethyl alcohol. A current of chlorine gas is passed through cooled absolute alcohol until it is saturated and attains a known specific gravity; the temperature is slowly raised to about 90° to complete the reaction. The crystals of chloral alcoholate formed on cooling are separated and distilled with conc. sulphuric acid. The reaction takes place in several stages. The chlorine first oxidizes the alcohol to acetaldehyde and the excess of alcohol combines with the aldehyde to give an alcoholate:



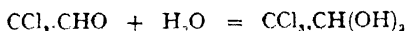
The alcoholate is chlorinated giving chloral alcoholate, $\text{CCl}_3\text{CH(OH)OC}_2\text{H}_5$, which is finally decomposed by sulphuric acid to give chloral:



Properties.—Chloral is a colourless, heavy, oily liquid with a pungent smell. It boils at 98.1° and has a sp. gr. of 1.512 at 20° . It is soluble in ether and chloroform. Like aldehydes it acts as a powerful reducing agent. It is chiefly used for the preparation of the hypnotic chloral hydrate and the insecticide D.D.T. (see p. 277).

Chloral Hydrate, $\text{CCl}_3\text{CH}(\text{OH})_2$

When chloral (m.w. 147.41) is shaken with $\frac{1}{8}$ th by weight or $\frac{1}{5}$ th by volume of water there is an evolution of heat and the mixture solidifies to a crystalline mass of chloral hydrate:



The reaction is peculiar since two hydroxyl groups are attached to the same carbon atom and gives a stable compound and this is probably due to the effect of the strongly electro-negative chlorine atoms.

Chloral hydrate is a colourless crystalline solid with a characteristic pungent smell and a sharp taste and melts at about 57° . It is soluble in water and alcohol. Like chloral it does not give the aldehyde reaction with Schiff's reagent. On heating with conc. sulphuric acid chloral is regenerated from chloral hydrate. By heating chloral hydrate with caustic soda, pure chloroform can be obtained:



Chloral hydrate is used in medicine as a hypnotic. For tests, see toxicology of chloral hydrate (see p. 472—4).

Butyl chloral hydrate, Trichlor butyraldehyde hydrate, $\text{CH}_3\text{CHCl.CCl}_3\text{CH}(\text{OH})_2$: this is a crystalline substance, m.p. 78° , used in medicine as a hypnotic and analgesic. It is prepared by passing chlorine through paraldehyde.

B. Unsaturated Aldehydes.

Acrolein, $\text{CH}_2=\text{CH}.\text{CHO}$: formed when fats and oils are overheated: prepared by distilling glycerol with KHSO_4 ; colourless liquid with a pungent odour; irritates the mucous membrane of the eyes; b.p. 52° ; soluble in 2 to 3 parts of water.

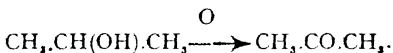
Crotonaldehyde, Methyl acrolein, $\text{CH}_3\text{CH}=\text{CH}.\text{CHO}$: prepared by heating alcohol with dehydrating agents: colourless pungent liquid, b.p. 105° ; soluble in water.

Citronellal, $\text{CH}_3=\text{C}(\text{CH}_3).\text{CH}_2.\text{CH}_2.\text{CH}_2.\text{CH}(\text{CH}_3).\text{CH}_2.\text{CHO}$: found in citronella oil and in other essential oils; pleasant smelling oily liquid, insoluble in water; b.p. 204° .

Citral, $(\text{CH}_3)_2\text{C}=\text{CH}.\text{CH}_2.\text{CH}_2.\text{C}(\text{CH}_3)=\text{CH}.\text{CHO}$: found in lemon grass oil and in other essential oils; pleasant smelling oily liquid, insoluble in water; b.p. 229° .

KETONES

We have seen that in an aldehyde $R-\overset{\text{H}}{\underset{\text{O}}{\text{C}}}$ a carbonyl group is attached to a hydrogen atom and to an alkyl radical. If the hydrogen atom is replaced by another alkyl group we get a ketone $R-\overset{\text{R}}{\underset{\text{O}}{\text{C}}}$. These ketones are obtained by the oxidation of secondary alcohols just as aldehydes are obtained by the oxidation of primary alcohols.



A ketone containing two similar alkyl radicals is called a *simple ketone* and one containing two dissimilar alkyl radicals is called a *mixed ketone*. The characteristic group of a ketone is thus the carbonyl group attached to two alkyl

radicals $\begin{matrix} \text{R} \\ \text{R} \end{matrix} \text{CO}$, where R may be similar or dissimilar. The

ketones exhibit a kind of structural isomerism known as *metamerism* in which different radicals are attached to the same multivalent atom or group. They have the same molecular formula but different structural formulæ, and are confined to compounds of the same class. Thus di-n-propyl ketone $\text{C}_3\text{H}_7-\text{CO}-\text{C}_3\text{H}_7$ and di-isopropyl ketone

$\begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix} \text{CH} \cdot \text{CO} \cdot \text{CH} \begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$ or methyl propyl ketone $\text{CH}_3-\text{CO}-\text{C}_3\text{H}_7$ and diethyl ketone $\text{C}_2\text{H}_5\text{CO}\cdot\text{C}_2\text{H}_5$ form *metamers*.

Nomenclature of Ketones.

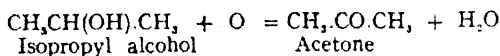
Usually, the suffix *ketone*, is added to the alkyl groups present: e.g., dimethyl ketone $\text{CH}_3\text{CO}\cdot\text{CH}_3$, methyl ethyl ketone $\text{CH}_3\text{CO}\cdot\text{C}_2\text{H}_5$, and so on. In the international system of nomenclature the suffix *-one* is added to the corresponding hydrocarbon after dropping the ending *-e* and putting in a numeral to show the position of the ketone group.

Thus, CH_3COCH_3 is propanone, $\text{CH}_3\text{CH}_2\text{COCH}_3$ is butan-2-one, $\text{CH}_3\text{CH}_2\text{CH}_2\text{COCH}_3$ is pentan-2-one, $\text{CH}_3\text{CH}_2\text{CH}_2\text{COCH}_2\text{CH}_3$ is hexan-3-one, and so on.

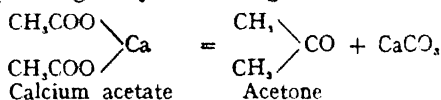
General Methods of Preparation.

(1) Oxidation of the corresponding secondary alcohols:

e.g.,

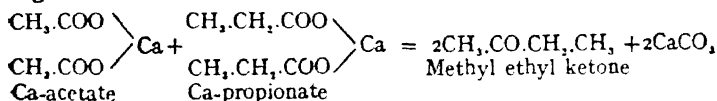


(2) Dry distillation of the calcium or barium salts of the corresponding fatty acids: e.g.,



If a mixture of two calcium salts is used, a mixed ketone, along of course with the two pure ketones, can be obtained:

e.g.



General Properties of Ketones and their Distinction from Aldehydes.

The simple ketones have the *general formula* R.CO.R where R is an alkyl radical, and they form the following *homologous series*:

Dimethyl ketone CH_3COCH_3 (b.p. 56.3°),

Diethyl ketone $\text{C}_2\text{H}_5\text{CO.C}_2\text{H}_5$ (b.p. 102.7°),

Dipropyl ketone $\text{C}_3\text{H}_7\text{CO.C}_3\text{H}_7$ (b.p. 144°),

Dibutyl ketone $\text{C}_4\text{H}_9\text{CO.C}_4\text{H}_9$.

Diamyl ketone $\text{C}_5\text{H}_{11}\text{CO.C}_5\text{H}_{11}$ (b.p. 226°),

and so on.

The lower members are all colourless liquids with characteristic smell and the higher ones are solids.

The ketones are not so reactive as the aldehydes. They do not give any red colour with Schiff's reagent and they do not reduce an ammoniacal solution of silver nitrate. But like aldehydes they form additive compounds with sodium bisulphite and hydrocyanic acid, and yield crystalline derivatives with hydroxylamine, phenyl hydrazine and semi-

carbazine (p. 123). The ketones do not yield polymers but form condensation products. They are reduced to secondary alcohols whereas the aldehydes are reduced to primary alcohols. On oxidation ketones break down to acids with a lower number of carbon atoms, while the aldehydes yield acids with the same number of carbon atoms.

Acetone, Dimethyl ketone, Propanone, $\text{CH}_3\cdot\text{CO}\cdot\text{CH}_3$.

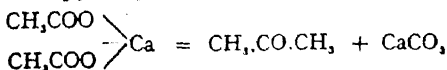
Occurrence and Formation. Acetone is present in traces in normal blood and urine. In pathological conditions like diabetes mellitus the amount increases considerably in the urine (acetonuria) as well as in the blood (acetonæmia). The presence of the so-called *acetone bodies* (acetoacetic acid $\text{CH}_3\text{CO}\cdot\text{CH}_2\text{COOH}$, β -hydroxy butyric acid $\text{CH}_3\text{CHOH}\cdot\text{CH}_2\text{COOH}$ and acetone), found in diabetic urine, is attributed to the incomplete combustion of fats in the body (see Chapt. 15, p. 182). Acetone is also found amongst the products of the dry distillation of wood and is formed during the fermentation of starch by special organisms.

Preparation.

(1) *In the dry distillation of wood*, acetone is found along with methyl alcohol. It has been mentioned before that *pyroligneous acid* contains only about 0.5 per cent of acetone and the *wood spirit* obtained from this contains from 10 to 20 per cent of acetone. The liquid obtained after separating the solid compound of methyl alcohol with CaCl_2 (see p. 77) is distilled and gives acetone.

The acetone still contains some methyl alcohol and is purified by treating the liquid with a strong solution of sodium bisulphite. The crystalline bisulphite compound is separated from the liquid impurities and dried. It is then decomposed by heating with a solution of sodium carbonate when acetone distils over. It is dried with anhydrous calcium chloride and redistilled.

(2) Acetone is mostly obtained by *heating calcium acetate* at 300° - 400° in the absence of air, the calcium acetate being obtained from pyroligneous acid and from other sources.



The liquid is fractionally distilled and finally purified as before through the bisulphite compound.

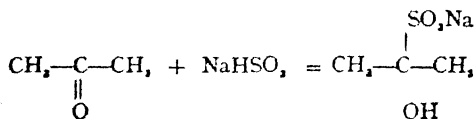
(3) *Biological Process*.—Acetone is also produced by the fermentation of starch obtained from rice, maize, potato, etc., with organisms such as *Clostridium acetobutylicum* and *Fernbach's Bacillus butylicus*. There is an yield of 15 to 25 per cent of acetone along with about twice the volume of n-butyl alcohol (b.p. 117°) and the acetone is purified by fractional distillation.

Properties and Reactions.

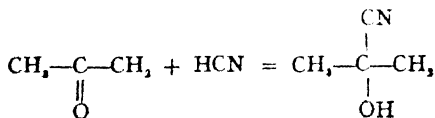
Acetone is a colourless mobile liquid with a peculiar smell and a burning taste. It has a sp. gr. of 0.792 at 20° and boils at 56.3° (760 m.m.). It mixes with water, alcohol and ether in all proportions. It is a good solvent for fats, resins, etc.

Acetone does not give any red colour with Schiff's reagent like aldehydes and it does not reduce an ammoniacal solution of silver nitrate except on prolonged boiling.

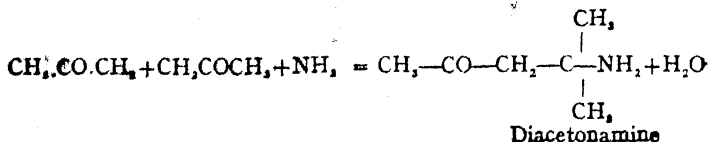
Like acetaldehyde acetone forms *additive compounds* such as *acetone bisulphite* with NaHSO_3 ,



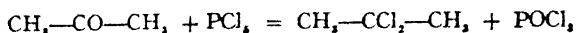
and *acetone cyanhydrin* with hydrocyanic acid,



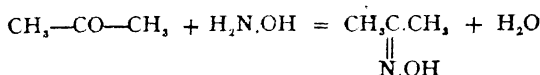
It does not, however, produce an additive compound with ammonia but there is a condensation reaction and *diacetoneamine* is formed:



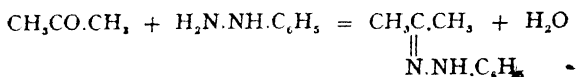
With PCl_5 acetone gives *dichloroacetone* or $\beta\beta$ -dichloropropane, *i.e.*, one atom of oxygen is replaced by 2 atoms of chlorine, indicating that there is no OH group:



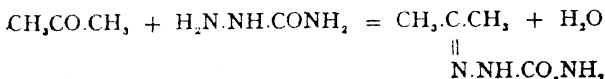
With hydroxylamine, acetone gives *acetoxime* (cf. aldoxime):



With phenylhydrazine, acetone gives *acetone phenylhydrazone*:

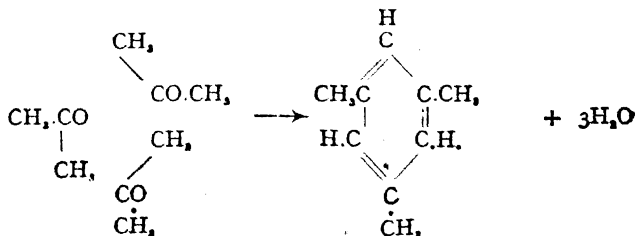


With semicarbazide, acetone gives *acetone semicarbazone*:



These oximes, phenylhydrazones and semicarbazones are crystalline compounds with characteristic melting points and serve for the identification of ketones.

When treated with conc. H_2SO_4 , 3 molecules of acetone condense together and give *mesitylene* or 1:3:5 trimethyl benzene. This is an example of the transformation of an open chain compound to a cyclic one:



On oxidation, acetone gives acetic acid, an acid with fewer carbon atoms, and carbon dioxide:



Uses of Acetone. It is largely used as a solvent in industries for varnishes, celluloid, collodion, smokeless powder, synthetic rubber, and for storing acetylene (see p. 62). It is also used in bacteriological work and in the preparation of some important drugs such as chloroform, iodoform, sulphonal, chloretone, eucaine, etc.

Tests for Acetone.

(1) On adding 5 drops of a freshly prepared 10 per cent solution of sodium nitroprusside and 1 c.c. of a 15 per cent solution of NaOH, there is a ruby red or reddish yellow colour, which on acidification with a few drops of glacial acetic acid becomes rose violet or wine red (Legal's Test).

(2) To the solution of acetone saturated with solid ammonium chloride or sulphate, add a few drops of freshly prepared solution of sodium nitroprusside, mix and then layer with some strong ammonia, when a purple or permanganate colour is formed at the junction which gradually spreads through the layers (Rothera's Test). If only a trace of acetone is present, it takes a few minutes to develop the ring. This test is also given by acetoacetic acid (see p. 178).

(3) **Iodoform Reaction.** The diluted solution of acetone is mixed with an equal amount of ammonia and a solution of iodine in potassium iodide is added drop by drop until there is a black precipitate of *nitrogen iodide*. On warming, the precipitate disappears and iodoform is produced. If carefully carried out this reaction serves to distinguish acetone from ethyl alcohol (see p. 89).

SOME HIGHER KETONES

Methyl ethyl ketone, Butan-2-one, $\text{CH}_3\text{CH}_2\text{COCH}_3$; occurs in crude wood spirit; liquid with ethereal smell, b.p. 78.6° ; used in the preparation of trional and in the manufacture of plastics.

Methyl-n-amyl ketone, Heptan-2-one, $\text{CH}_3(\text{CH}_2)_4\text{COCH}_3$; occurs in clove oil and in other essential oils; oily liquid with penetrating odour; b.p. 150° .

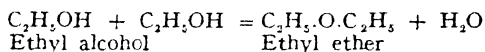
Methyl-n-nonyl ketone, Undecan-2-one, $\text{CH}_3(\text{CH}_2)_8\text{COCH}_3$; occurs in oil of rue; oily liquid with orange like odour; b.p. 230° .

CHAPTER XI

ETHERS, THIO-ALCOHOLS AND THIO-ETHERS

ETHERS

Just as an alcohol (*e.g.*, C_2H_5OH) may be regarded as the hydroxide of an alkyl radical (C_2H_5), an ether (*e.g.*, $C_2H_5.O.C_2H_5$) may be regarded as the oxide of two alkyl radicals. Ethers may also be considered as *anhydrides* of alcohols since they are produced by the elimination of water from two molecules of alcohol: thus,



If the two alkyl radicals are the same, the ether is known as a *simple ether* (*e.g.*, $CH_3.O.CH_3$, $C_2H_5.O.C_2H_5$, etc.) while if the two radicals are different the ether is called a *mixed ether* (*e.g.*, $CH_3.O.C_2H_5$, $C_2H_5.O.C_3H_7$, etc.). The *general formula* of a simple ether will thus be $R.O.R$ and for a mixed ether $R.O.R'$, where R and R' are different alkyl groups. Like the ketones, ethers exhibit the type of isomerism known as *metamerism*. Thus, diethyl ether $C_2H_5.O.C_2H_5$ (or $C_4H_{10}O$) and methyl propyl ether $CH_3.O.C_3H_7$ (or $C_4H_{10}O$) or ethyl propyl ether $C_2H_5.O.C_3H_7$ (or $C_5H_{12}O$) and methyl butyl ether $CH_3.O.C_4H_9$ (or $C_5H_{12}O$) form *metamers*.

Nomenclature of Ethers: As is clear from the above examples, the ethers are designated by the names of the alkyl groups attached to the oxygen atom.

General Properties and Reactions.

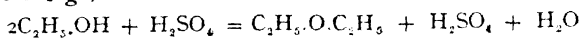
The *simple ethers* form the following *homologous series*:

Dimethyl ether $CH_3.O.CH_3$ (gas, b.p. -23.6°)
Diethyl ether $C_2H_5.O.C_2H_5$ (liquid, b.p. 34.6°)
Dipropyl ether $C_3H_7.O.C_3H_7$ (liquid, b.p. 90.7°)
Diethyl ether $C_4H_9.O.C_4H_9$ (liquid, b.p. 140.9°)
etc., etc.

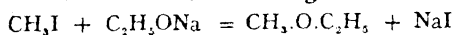
The lowest member, dimethyl ether, is a gas, the higher members are colourless mobile liquids with characteristic odour, and only the very highest ones like cetyl ether $C_{16}H_{33}.O.C_{16}H_{33}$, are solids. They are all lighter than water and their boiling points are lower than those of the corresponding alcohols. They are almost insoluble in water but dissolve easily in alcohol, chloroform, benzene and petroleum ether. They are neutral inert substances having no action upon metallic sodium, phosphorus pentachloride or alcoholic potash.

General Methods of Preparation.

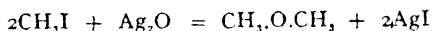
(1) By the action of concentrated sulphuric acid upon an alcohol: *e.g.*,



(2) By the action of an alkyl iodide upon sodium alcoholate in alcoholic solution: *e.g.*,



(3) By the action of an alkyl iodide upon silver oxide: *e.g.*,



Ether, *Diethyl Ether, Sulphuric Ether, Ethyl Oxide*, $C_2H_5.O.C_2H_5$.

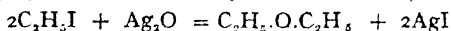
The name 'sulphuric' ether originated from an old erroneous belief that it contained sulphur. The name is still retained in common use since sulphuric acid is used in its preparation and it serves to distinguish this from other ethers, specially from petroleum ether.

Synthesis.

(1) By the action of ethyl iodide upon sodium ethoxide in alcoholic solution:



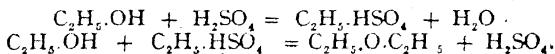
(2) By the action of ethyl iodide upon silver oxide:



Preparation.

In the laboratory as well as on a large scale, ether is made by the *continuous etherification process*. The method consists in dehydrating ethyl alcohol with concentrated

sulphuric acid. Sulphuric acid, acting upon the alcohol, first produces ethyl hydrogen sulphate. The ethyl hydrogen sulphate acting upon fresh alcohol produces ether and regenerates sulphuric acid:



The sulphuric acid again acts upon fresh alcohol and the process is thus a continuous one. In practice, however, one part of concentrated sulphuric acid can convert only about 10 parts of alcohol into ether since the acid gets diluted with the water formed and the reverse reaction occurs. There are also side reactions like the formation of ethylene or the partial reduction of sulphuric acid to form SO_2 .

A mixture of rectified spirit (50 c.c.) and conc. sulphuric acid (50 c.c.) is taken in a round bottomed distillation flask (cap. 500 c.c.) containing some clean dry sand, to ensure

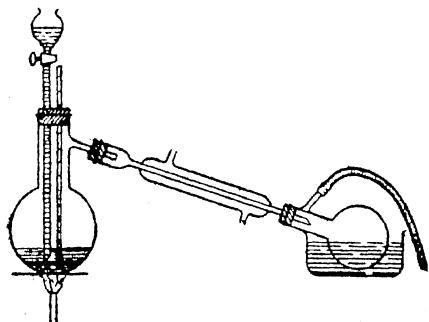


FIG. 27.

steady boiling, and fitted with a long thermometer and a tap funnel both of which dip into the mixture of alcohol and acid. The side tube of the flask is attached to a long condenser through which ice water is circulated. The condenser is attached to another small distillation flask which acts as the receiver (Fig. 27). The receiver is kept cool with ice water and the side tube is connected with a long rubber tube leading to a sink so that the inflammable vapours of ether do not come in contact with a flame. The flask is heated over a sand bath and the temperature of the mixture of alcohol and acid is kept between 140° - 145° . The rectified spirit (50 c.c.) kept in the tap funnel is then allowed to flow drop by drop at the same rate at which ether distils over, until the reaction is complete. The distillate, containing ether with water, alcohol, sulphur

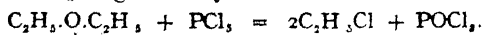
dioxide, aldehydes and other impurities, is taken in a separating funnel, washed first with dilute caustic soda to remove the sulphur dioxide and then with a strong solution of common salt to remove the alcohol. The ether is then dried with anhydrous calcium chloride, filtered and redistilled.

To obtain perfectly *anhydrous ether*, it is first shaken with anhydrous calcium chloride, filtered and then treated with finely drawn wires of metallic sodium, and redistilled.

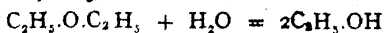
Ether prepared from methylated spirit is known as *methylated ether*, which is used for commercial purposes as a solvent, etc.

Properties and Reactions.

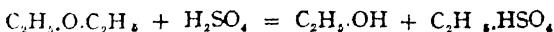
Ether is a mobile colourless liquid with a characteristic odour. It has a sp. gr. of 0.720 at 15° and boils at 34.6° (760 mm.). It is very volatile and produces intense cold by its rapid evaporation. Its vapour is highly inflammable and is about $2\frac{1}{2}$ times heavier than air and when heated its vapour flows down instead of rising up as in the case of petroleum ether. Ether vapour forms an explosive mixture with air, oxygen or nitrous oxide in certain proportions. The concentration necessary for explosion with air is stated to be between 1.8 and 6.0 per cent of ether vapour by volume, the optimum concentration being about 4 per cent. Below the lower limit, the mixture neither explodes nor takes fire as it is too much diluted with air. Above the upper limit the mixture becomes inexplodable but remains inflammable all the same. Being very inflammable, ether should always be kept away from a naked flame. Ether mixes with absolute alcohol, chloroform or petroleum ether in all proportions. 100 c.c. of water dissolve about 8.1 c.c. of ether at 22° and 100 c.c. of ether will dissolve about 2.9 c.c. of water at 22°. Ether is a very inert liquid having no action on alkalis, metallic sodium or cold PCl_5 . When heated with PCl_5 it gives ethyl chloride:



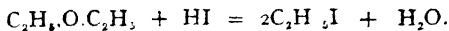
Heated with water under pressure in the presence of H_2SO_4 it yields alcohol:



It yields alcohol and $C_2H_5.HSO_4$ when heated with conc. H_2SO_4 :



Concentrated HI decomposes ether on heating and yields ethyl iodide:



Exposure to air and sunlight is stated to bring about a partial decomposition of ether with the formation of an unstable compound, vinyl alcohol $CH_2:CH.OH$ and hydrogen peroxide, both of which impart to the sample the property of exploding by heat, percussion or violent shock. Similarly, oxygen, ozone and certain substances known as carriers of oxygen oxidize ether in the presence of moisture to a poisonous and highly explosive substance *ethyl peroxide*, $\begin{matrix} C_2H_5 \\ C_2H_5 \end{matrix} > O=O$, which explodes readily on heating, especially in the presence of fats and oils and other organic substances. For these reasons, ether should always be stored in a dark place in amber coloured bottles filled up to the neck, specially when required for anæsthetic purposes. It is also desirable to use small bottles of ether for anæsthesia in the operation theatre and to reject the remnants in the bottles after the day's work instead of transferring the same to other bottles for next day's operations. In the presence of oxygen ether is readily decomposed by red hot platinum wire and perhaps also by other red hot metals into acetaldehyde, formaldehyde, etc. Electro-cautery in nose or throat under ether anæsthesia is, therefore, attended by a certain amount of risk to the patient.

Uses.—Ether is an excellent solvent for fats and oils and is also a good solvent for resins, collodion, alkaloids, etc. It is, therefore, used widely as a solvent both in the laboratory and in industry. Ether is used as an anæsthetic and although it is weaker than chloroform in its action it is preferred to the latter since it is less toxic. For light operative anæsthesia, the concentration of ether vapour in air is about 5.6 per cent by volume or 18.5 milligrams of ether per 100 c.c. of air. It proves fatal if the concentration goes up to 11 per cent.

The concentration of ether vapour under the mask during surgical operations usually averages to 7 per cent by volume.

Impurities of Ether and their Tests.

The impurities likely to be present are alcohol, sulphurous acid and other free acids, aldehydes and ketones, ethyl peroxide, etc. The B.P. ether for anaesthesia should be free from all these impurities.

(1) *Test for peroxide*.—Take 8 c.c. of 10 per cent KI and 5 drops of a freshly prepared solution of starch in a stoppered tube, fill to the brim with the ether, stopper without allowing any air bubble, shake vigorously and set aside in the dark for half an hour. A brown or reddish colour is produced if traces of peroxide are present.

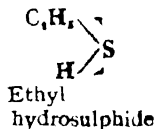
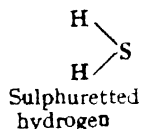
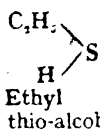
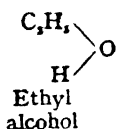
(2) *Test for acetone and aldehydes*.—Take 2 c.c. of Neseler's reagent in a stoppered tube (12 c.c.) and fill the tube with the ether. Insert the stopper, shake vigorously for a few seconds and set aside for 5 minutes. No colour or turbidity is produced if ether is free from acetone or aldehydes.

Alcohol, SO_2 , etc., are detected by the usual tests.

N.B. *Hydroquinone* in a concentration of 1 in 5000 is sometimes added to ether to prevent the formation of peroxide. Its presence vitiates the test for aldehydes and it should, therefore, be separated by distillation before the test is applied. Other antioxidants such as *pyrogallol* and *propyl gallate* are also used for this purpose.

THIO-ALCOHOLS OR MERCAPTANS

The thio-alcohols may be considered to be sulphur analogues of the alcohols. They may also be regarded as derivatives of sulphuretted hydrogen, one atom of hydrogen of which has been replaced by an alkyl group; they are, therefore, also known as *alkyl hydrosulphides*.



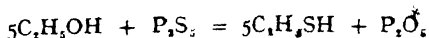
The thio-alcohols are also known as mercaptans (*mercurium-mercury, captans*—seizing) from the fact that they react easily with mercuric oxide to form crystalline precipitates of mercury mercaptides, e.g., $(\text{C}_2\text{H}_5\text{S})_2\text{Hg}$.

General Methods of Preparation.

(1) By warming an alkyl halide with potassium hydrosulphide in alcoholic solution:

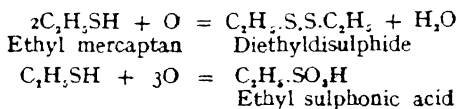


(2) By heating an alcohol with phosphorus penta-sulphide, thus replacing O by S:



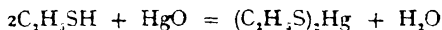
General Properties and Reactions.

The thio-alcohols are colourless liquids, except methyl mercaptan which is a gas. The lower members have an extremely nauseating smell. The boiling points of thio-alcohols are lower than those of the corresponding alcohols, e.g., $\text{C}_2\text{H}_5\text{SH}$ boils at 37° . They are insoluble in water but soluble in aqueous alkalis. They dissolve in ether and alcohol. Like alcohols the mercaptans react with metallic K or Na with evolution of hydrogen and the formation of K or Na salts which are easily decomposed by water. The mercaptans are easily oxidized. Thus air oxidizes them to disulphides and nitric acid converts them to sulphonic acids:



The former reaction, viz., the oxidation of a thio-alcohol to a disulphide, is one of much biochemical interest and is found to occur in the living cell, e.g., in the transformations of cysteine to cystine or of glutathione to a disulphide (see Chapters on Proteins.).

Mercaptans react readily with mercury salts, or even with mercuric oxide, to form crystalline mercaptides:

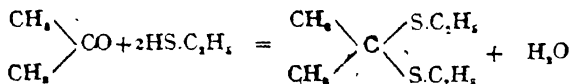


Methyl Mercaptan, Methane Thiol, CH_3SH : a product of putrefaction of proteins; found in intestinal gases and sometimes in urine; gas with unpleasant smell, b.p. 5.8° (752 mm.).

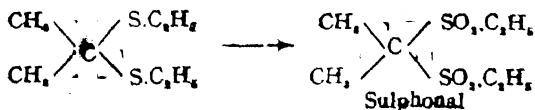
Ethyl Mercaptan, Ethane Thiol, $\text{C}_2\text{H}_5\text{SH}$: liquid with unpleasant smell; very little soluble in water, b.p. 37° (760 mm.); sp. gr. 0.839 at 20° .

Sulphonal, or Diethyl sulphone dimethyl methane:

Acetone reacts with ethyl mercaptan giving acetone mercaptol:

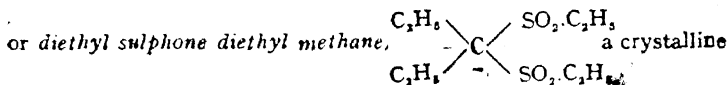


If this mercaptol is oxidized with a solution of potassium permanganate diethyl sulphone dimethyl methane or sulphonal is formed:



Sulphonal is a crystalline solid, m.p. 126° , and is used in medicine as a hypnotic (soporific).

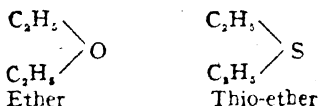
Trional or *Diethyl sulphone methyl ethyl methane*, is a similar crystalline compound, m.p. 76° , prepared from ethyl mercaptan and methyl ethyl ketone. It has a more powerful and prolonged hypnotic action than sulphonal. Another such compound, **tetrona**



solid, m.p. 89° , is prepared from ethyl mercaptan and diethyl ketone. This compound is very insoluble in water and therefore not so good as a hypnotic for human beings, but it has a very powerful action on dogs. The physiological activity of these types of sulphones (see later) seems to depend upon the ethyl groups attached to sulphur.

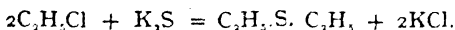
ALKYL SULPHIDES OR THIO-ETHERS

These may be considered as derivatives of sulphuretted hydrogen the hydrogen atoms being replaced by alkyl groups, or as ethers in which the oxygen atom is replaced by sulphur; hence the names:



General Methods of Preparation.

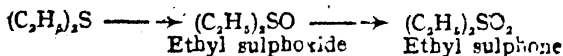
(1) Action of alkyl halides upon potassium sulphide:



(2) Action of phosphorus pentasulphide upon ethers.



General Properties and Reactions. The alkyl sulphides are volatile liquids with a disagreeable smell. They are insoluble in water but soluble in alcohol and ether. They are neutral in reaction. On mild oxidation they are converted into *sulphoxides* and on stronger oxidation to *sulphones*, which are stable crystalline substances:

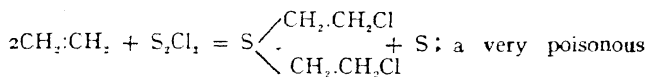


Dimethyl Sulphide, $\text{CH}_3\cdot\text{S}\cdot\text{CH}_3$; occurs in petroleum from Ohio and in some samples of geranium oil; liquid with horse-raddish like odour; b.p. 38° .

Diethyl Sulphide, $\text{C}_2\text{H}_5\cdot\text{S}\cdot\text{C}_2\text{H}_5$; liquid with ethereal smell; b.p. 92° ; insoluble in water.

Mustard Gas, *$\beta\beta'$ -Dichlorodiethyl sulphide*,
 $\text{Cl}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{Cl}$:

prepared by the action of sulphur monochloride on ethylene:



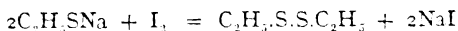
oily, volatile, colourless liquid with a faint horse-raddish or mustard like odour; sp. gr. 1.27; boils with decomposition at 217° , volatile with steam. It is almost insoluble in water but is soluble in kerosene, alcohol, ether, carbon tetrachloride, glycerol and all vegetable oils and fats. It is slightly soluble in vaseline. It penetrates through leather and wood work and is absorbed by rubber; causes painful blisters on the skin, and extensive damage in the lung tissue when inhaled; used as a poison (blistering or vesicant) gas in the first Great War; a concentration of 0.07 mg. per litre of air proves fatal on exposure for 30 minutes. It is 5.5 times heavier than air; it is slowly hydrolyzed by water into HCl and $\text{S}(\text{CH}_2\text{CH}_2\text{OH})_2$ (thiodiglycol) which is not toxic. Bleaching powder, which is used as an antidote, reacts violently and decomposes mustard gas with evolution of much heat and formation of CO_2 , HCl , chloroform, chloral, etc., which are all harmless. So much heat is evolved in this reaction that the patient gets severe burns if undiluted bleach is used.

ALKYL DISULPHIDES

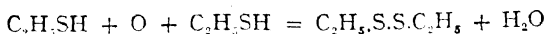
Preparation.

(1) By the action of iodine upon sodium mercaptides;

e.g.,



(2) By the oxidation of a mercaptan; *e.g.*,



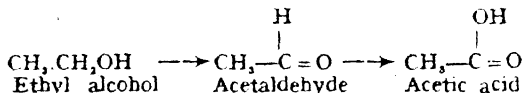
Diallyl disulphide, $(\text{CH}_2\text{:CH}\cdot\text{CH}_2)_2\text{S}_2$; occurs in oil of garlic as its chief component; liquid with odour of garlic. b.p. $78\text{--}80^\circ$ (16 mm.).

Allyl propyl disulphide, $\text{CH}_2\text{:CH}\cdot\text{CH}_2\cdot\text{S}\cdot\text{S}\cdot\text{C}_2\text{H}_5$; occurs in oil of garlic; b.p. $66\text{--}69^\circ$ (16 mm.); liquid with odour of onions.

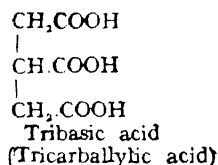
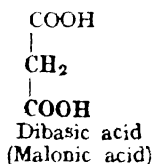
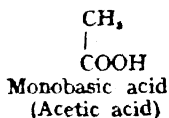
CHAPTER XII

MONOBASIC CARBOXYLIC OR FATTY ACIDS, SATURATED

We have seen before that a primary alcohol, the characteristic group of which is CH_2OH , when carefully oxidized, yields an aldehyde, the CH_2OH group changing to CHO . If an aldehyde is further oxidized we get an acid and the CHO group is oxidized to COOH , which is



the *characteristic group* of an organic acid. It is monovalent and is known as the *carboxyl group*. The H atom of the carboxyl group is acidic and is replaceable by a metal forming a salt or by an alkyl radical to form what is called an *ester*. The acid is said to be *monobasic* if there is only one carboxyl group, *dibasic* if there are two carboxyl groups, and so on. Thus, the basicity does not depend on the number of H atoms as in inorganic acids but on the number of carboxyl groups. Ordinary organic acids are called carboxylic acids on account of the presence of acid COOH group, but there are organic compounds which act as acids and form salts but do not contain any COOH group, *e.g.*, uric acid, carbolic acid, etc.



The saturated monobasic acids form the following *homologous series*, and possess the *general formula* $\text{C}_n\text{H}_{2n+1}\text{COOH}$:—

Formic acid $\text{H}\cdot\text{COOH}$ (b.p. 100.8°)

Acetic acid $\text{CH}_3\cdot\text{COOH}$ (b.p. 118°)

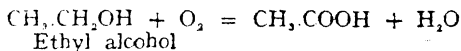
Propionic acid $C_2H_5.COOH$ (b.p. 141°)
 n-Butyric acid $C_3H_7.COOH$ (b.p. 162.5°)
 n-Valeric acid $C_4H_9.COOH$ (b.p. 185.4°)
 n-Caproic acid $C_5H_{11}.COOH$ (b.p. 205°)
 etc., etc.
 Palmitic acid $C_{15}H_{31}.COOH$ (m.p. 62.5°)
 Stearic acid $C_{17}H_{35}.COOH$ (m.p. 69.5°)
 etc., etc.

The monobasic acids of this series are known as *fatty acids* since some of the higher members are present in fats as esters.

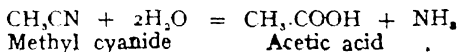
Nomenclature of Acids.—Although some of the acids have retained their old special names, it is usual to designate them according to the number of carbon atoms in the molecule: *e.g.*, hexoic acid, nonylic acid, etc. According to the Geneva nomenclature, the word 'acid' is attached to the hydrocarbon from which it is derived. Thus formic acid is methane acid, acetic acid is ethane acid, n-valeric acid is pentoic acid, stearic acid is octadecylic acid, and so on. In describing the position of a substituent in an organic acid, the carbon atom adjoining the $COOH$ group is called the α carbon atom, the next one the β carbon atom, and so on. Thus, $CH_3.CH_2.CHCl.COOH$ is α -chloro butyric acid, $CH_3.CH(OH).CH_2.COOH$ is β -hydroxybutyric acid, and so on.

General Methods of Preparation.

(1) Oxidation of the corresponding primary alcohols:
e.g.,



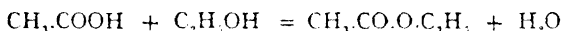
(2) Hydrolysis of alkyl cyanides: *e.g.*,



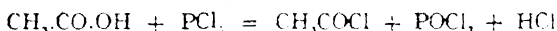
General Properties of Acids.

The members of this series up to capric acid $C_{10}H_{20}O_2$ are volatile with steam and these are, therefore, called *volatile fatty acids*. The lower members (up to pelargonic acid $C_9H_{18}O_2$, m.p. 12.5°) are colourless liquids with characteristic

smell but the higher members (from capric acid, m.p. 31.5°) are colourless crystalline solids and are greasy to the touch. The lower acids are easily soluble in water but the solubility decreases rapidly with increase of molecular weight. They are all soluble in alcohol and ether. The aqueous and alcoholic solutions turn blue litmus red. The hydrogen atom of the carboxyl group is replaceable by metals with the formation of salts. The sodium, potassium or ammonium salts are generally soluble in water but the calcium and magnesium salts of the higher acids are insoluble (see hard water). They combine with alcohols forming esters, e.g.,



The acids react with phosphorus pentachloride with the formation of acid chlorides: e.g.,



With the exception of formic acid the acids are fairly stable towards the common oxidizing agents although very strong oxidizing agents would ultimately convert them into CO_2 and H_2O .

Formic Acid, Methane Acid, H.CO.OH

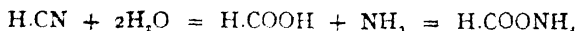
Occurrence.—It is found in red ants (*Formica rufa*, from which the name 'formic' is derived), in the stings of some insects, in the hair or bristles of the common nettles (*Bichuti* plant) *Tragia involucrata*, and in small quantities in perspiration and human urine. It has also been found in the fruit juices of many plants.

Synthesis.

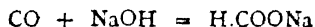
(1) By the oxidation of formaldehyde:



(2) By the hydrolysis of hydrocyanic acid:



(3) By the action of carbon monoxide, the anhydride of formic acid, on caustic soda, sodium formate is produced:

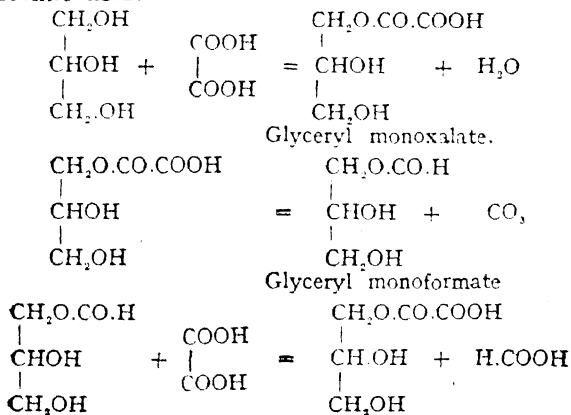


This method is utilized *on a commercial scale* in which CO is passed under pressure of 7-8 atmospheres over soda-lime heated to about 210° . The sodium formate formed is decomposed by heating with sodium bisulphate when 97-98 per cent of formic acid distils over.

Preparation.

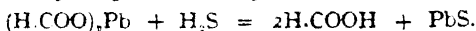
(4) *In the laboratory*, it is most conveniently prepared by heating anhydrous oxalic acid with anhydrous glycerol. A mixture of glycerol (50 c.c.) and oxalic acid (40 g.) is taken in a distilling flask connected with a water condenser and fitted with a thermometer which dips into the glycerol mixture. The flask is gradually heated over wire gauze until the temperature of the mixture lies between 110° - 120° . There is a vigorous evolution of carbon dioxide and formic acid gradually distils over. When the evolution of carbon dioxide subsides, the liquid is cooled down to 70° - 80° and more oxalic acid (40 g.) is added. The temperature is again raised to 110° - 120° and a further lot of formic acid is obtained.

The reaction takes place in three stages. An ester of glycerol, glyceryl monoxalate, is first formed. At the temperature of the reaction the monoxalate is decomposed with the evolution of carbon dioxide and the formation of glyceryl monoformate. The monoformate again acts on fresh oxalic acid with the formation of monoxalate and the production of formic acid:



N.B.—If the proportion of glycerol is doubled and the temperature is raised, allyl alcohol, an unsaturated alcohol (see p. 91) is formed.

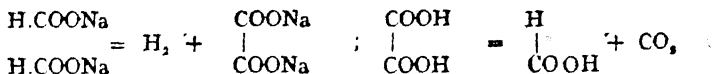
(5) *Anhydrous formic acid* can be obtained by passing sulphuretted hydrogen over dry heated lead formate:



Properties and Reactions.

Formic acid is a colourless liquid which blisters the skin and has a pungent odour. It boils at 100.8° (760 mm.) and melts at 8.6° . It has a sp. gr. of 1.22 at 20° . It mixes with water in all proportions and dissolves in alcohol and ether. It possesses a strong acid reaction yielding formates with carbonates, hydroxides, etc.

It is decomposed when warmed with conc. H_2SO_4 with the evolution of carbon monoxide and hence formic acid cannot be dehydrated by this means. Heating formic acid or a formate with conc. H_2SO_4 forms, however, a very good *laboratory method of preparing pure carbon monoxide*. Formic acid, H.CO.OH may be considered as the hydroxide of the *formyl radical* $\text{H.CO}-$, which is identical with the aldehyde group $-\text{CHO}$. In acetyl ($\text{CH}_3\text{CO}-$), propionyl ($\text{CH}_3\text{CH}_2\text{CO}-$) and other acid radicals of the higher homologues of formic acid, the H atom of the formyl radical is replaced by alkyl groups and the aldehyde group therefore disappears. Hence formic acid and its salts (formates) may be expected to possess reducing properties not shown by the higher fatty acids or their salts. A formate will thus reduce a solution of silver nitrate to metallic silver and a solution of mercuric chloride to mercurous chloride and then to metallic mercury. It will also decolorize an acid or alkaline solution of KMnO_4 . When a neutral ferric chloride solution is added to a neutral solution of a formate, a red colour due to the formation of ferric formate is produced. On heating this solution, a reddish brown precipitate of basic ferric formate $\text{Fe}(\text{OH})_2(\text{H.COO})$ is obtained. The lead salt and the silver salt are sparingly soluble in water while the other salts are easily soluble. If dry sodium formate is heated, sodium oxalate is formed with the liberation of hydrogen; on the other hand, if oxalic acid is heated, it yields formic acid and carbon dioxide:

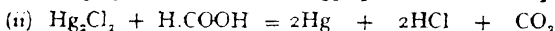
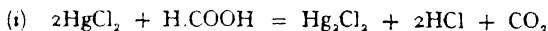


Tests

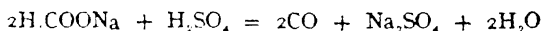
(1) *Micro-test*: If a neutral solution of a formate is treated with cerium nitrate and the solution allowed to evaporate, colourless aggregates of pentagonal dodecahedra are obtained.

(2) Neutral ferric chloride added to a neutral solution of a formate gives a red colour which is discharged by HCl. On boiling the red solution a reddish brown precipitate of the basic salt is obtained $[\text{H.COO.Fe(OH)}_2]$.

(3) On warming a formate solution with mercuric chloride there is a precipitate of mercurous chloride. If there is an excess of formate, the mercurous chloride is reduced to metallic mercury.



(4) Cold conc. H_2SO_4 when added to a formate liberates carbon monoxide which will burn with a blue flame.



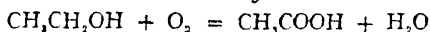
(5) *Silver mirror test*.—On warming a neutral solution of a formate with ammoniacal silver nitrate, a silver mirror or more usually a grey precipitate of metallic silver is formed.

Acetic Acid, Ethane Acid, CH_3COOH

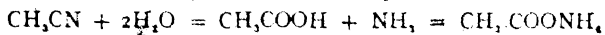
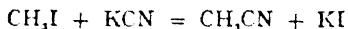
Occurrence. It is stated to be present in the free state or as a salt in minute amounts in muscle juice, in perspiration and in faeces. Salts or esters of acetic acid are known to be present in various plants *e.g.*, as glyceryl ester in croton oil, as linalyl acetate in oil of bergamot, etc. It is present in vinegar and is also formed during the fermentation of sugars.

Synthesis.

(1) By the oxidation of ethyl alcohol:

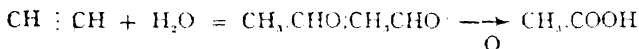
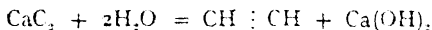


(2) By the hydrolysis of methyl cyanide which can be synthesized from methyl iodide:



Preparation of Acetic Acid.

(3) *From calcium carbide*: This synthetic method is now-a-days used for the manufacture of acetic acid. The acetaldehyde obtained is oxidized by heating it in a current of air in presence of a catalyst such as manganese acetate.



(4) *From destructive distillation of wood*: The *pyro-ligneous acid* obtained (see under methyl alcohol) is treated with milk of lime to neutralize the acetic acid and the mixture of methyl alcohol, acetone, etc., is distilled off. The calcium acetate obtained in the residue is dissolved in water, filtered from tarry impurities and treated with sodium sulphate. Calcium sulphate is precipitated and the solution of sodium acetate is filtered and evaporated to dryness. The residue is heated to fusion to remove water and to destroy some of the impurities and the dry anhydrous sodium acetate is taken in iron stills and decomposed with conc. H_2SO_4 and distilled. The distillate is fractionated in suitable stills and purified further by distillation after treatment with potassium dichromate which oxidizes organic impurities if any.

Vinegar.

Vinegar (Lat. *vinum*—wine, *acer*—sour) is the acid liquor obtained by the acetic fermentation of alcoholic liquids (such as wine, cider, beer, fermented malt solution or 'wort', etc., by *Mycoderma aceti* or "mother of vinegar", a fungus the spores of which are present in the air) and usually contains about 4-6 per cent of acetic acid. The vinegars possess different names according to their origin, e.g., white or *wine-vinegar* and brown or *malt-vinegar*, etc. Owing to the low content of acetic acid in vinegar (max. about 7 per cent), it is never used for the preparation of acetic acid. The maximum concentration of acetic acid in "acetic fermentation" of wines does not exceed 14 per cent.

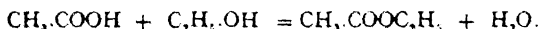
Quick-vinegar Process. A large wooden cask, provided with a perforated bottom and side holes to allow a free access of air, is filled with wood shavings which allow an increased surface for the exposure of the alcohol to air. The wood shavings are previously moistened with strong vinegar which contains the fungus *Mycoderma aceti*. Dilute alcohol (7-8 per cent) is then allowed to trickle slowly from above through a perforated wooden disc. The oxidizing enzyme secreted by the fungus oxidizes alcohol in the presence of air to acetic acid. The cask is kept at about 30° - 35° and the liquid collecting at the bottom is run through the shavings several times. Besides acetic acid, vinegar contains some alcohol, acids like tartaric acid and succinic acid and some esters, the aroma of the vinegar being due to the latter.

Properties and Reactions of Acetic Acid.

Pure acetic acid is a colourless liquid at ordinary temperature but solidifies at 16.7° to a white crystalline mass which looks like ice and from which the name '*glacial*' acetic acid has been given to the pure acid. It has a characteristic pungent odour and produces blisters on the skin. It boils at 118.1° (760 mm.) and has a sp. gr. of 1.0514 at 20° . It is hygroscopic and mixes with water, alcohol and ether in all proportions. It burns with a feebly luminous flame.

Acetic acid is stable towards oxidizing agents such as potassium dichromate or potassium permanganate and is sometimes used as a solvent for oxidizing organic substances. The normal salts of acetic acid are all soluble in water, the mercury and silver salts being sparingly soluble. It is an excellent solvent for organic substances and also dissolves sulphur, iodine and a few other inorganic substances.

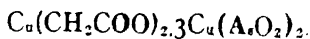
If dry chlorine is passed through glacial acetic acid in the presence of sunlight and some catalyst like iodine or red phosphorus, the three hydrogen atoms of the methyl group are gradually replaced by chlorine and we get *monochloroacetic acid* $\text{CH}_2\text{Cl.COOH}$, *dichloroacetic acid* CHCl_2COOH and *trichloroacetic acid* CCl_3COOH . When acetic acid is heated with some ethyl alcohol in presence of a little conc. H_2SO_4 , ethyl acetate is formed:



With phosphorus tri- or pentachloride acetic acid gives *acetyl chloride* CH_3COCl the OH group of COOH being replaced by chlorine.

Uses of Acetic Acid and its Salts.

Acetic acid is used as a solvent for organic substances and is a very common laboratory reagent. The glacial acid is used in medicine for destroying warts. Zinc dust and glacial acetic acid is a useful reducing agent. Acetic acid forms both normal and basic salts. *Calcium acetate* $(\text{CH}_3\text{COO})_2\text{Ca}$ is used in the manufacture of acetone. *Sodium acetate* $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ is used as a buffer and *ammonium acetate* $\text{CH}_3\text{COONH}_4$ is used in medicine as a diuretic. Normal or *neutral lead acetate* $(\text{CH}_3\text{COO})_2\text{Pb} \cdot 3\text{H}_2\text{O}$ is a colourless crystalline solid. It possesses a sweet taste and hence it is known as *sugar of lead*; it is however poisonous. It is used in medicine as an astringent. *Basic lead acetate* $\text{Pb}(\text{CH}_3\text{COO})_3 \cdot \text{PbO}$ is prepared by dissolving litharge PbO in a solution of normal lead acetate. A dilute solution of this salt is used in medicine under the name of *Goulard's lotion* as a cooling and astringent lotion in sprains and fractures. Both the normal and basic lead acetates are important reagents and used as precipitants particularly for the precipitation of tannins and colouring matters from solution. Basic copper acetate $(\text{CH}_3\text{COO})_2\text{Cu} \cdot \text{Cu}(\text{OH})_2$, known as *verdigris*, is used as a green pigment. A double salt of copper acetate and copper arsenite



known as *Schweinfurt green* or *Paris green*, is used as an insecticide, especially for killing mosquito larvæ as an antimalarial measure. The acetates of iron, aluminium and chromium are used as mordants in dyeing.

Tests for Acetic Acid.

(1) A neutral solution of ferric chloride gives with a neutral solution of acetic acid a deep red colour due to the formation of ferric acetate $(\text{CH}_3\text{COO})_3\text{Fe}$. The red colour is discharged by HCl . On boiling the red solution, a brown precipitate of basic ferric acetate $(\text{CH}_3\text{COO})\text{Fe}(\text{OH})_2$ is formed.

(2) When heated with alcohol and conc. H_2SO_4 , acetic acid produces ethyl acetate which is recognized by its characteristic pleasant odour.

(3) On heating a mixture of a dry acetate with a little arsenious oxide, an extremely nauseous odour of cacodyl oxide $(CH_3)_3As.O.As(CH_3)_3$ is perceived; this is extremely poisonous.

(4) Silver nitrate added to a neutral solution of acetic acid gives a white precipitate of silver acetate which is not reduced by boiling (distinction from formate).

(5) Micro-test: A few drops of uranyl formate (or uranyl nitrate + Na-formate) is added to a fairly concentrated solution of the acetate on a glass slide and the mixture allowed to evaporate slowly. On examining the residue on the edges of the cover slip under the microscope, very characteristic pale yellow tetrahedral crystals of sodium uranyl acetate are observed.

Some Common Saturated Fatty Acids.

Propionic Acid, $CH_3.CH_2.COOH$: found in pyroligneous acid, in sweat and in certain fermentation processes; liquid with pungent smell; b.p. 141° ; sp. gr. 0.9937 at 20° ; miscible with water in all proportions, but separates out as an oily liquid by addition of $CaCl_2$.

n-Butyric Acid, $CH_3.CH_2.CH_2.COOH$: found in the free state in small amounts in rancid butter and in perspiration; occurs as glyceryl ester, known as *butyrin*, to the extent of about 5 per cent in butter or *ghee* which is thus differentiated from body fat of animal and also from vegetable fats and oils; prepared by the *butyric fermentation* of carbohydrates induced by *Clostridium butyricus* and other bacteria; colourless liquid with a disagreeable smell like rancid butter; b.p. 162.5° ; sp. gr. 0.9587 at 20° ; miscible with water above -3.8° ; volatile with steam.

n-Valeric Acid, *Pentolic Acid*, $CH_3.(CH_2)_3.COOH$: found in pyroligneous acid; stated to be present as an ester in some species of valerian roots (*V. Wallichii* DC); colourless liquid with odour like butyric acid; b.p. 185.4° ; sp. gr. 0.9415 at 20° ; slightly soluble in water; volatile with steam.

Iso-Valeric Acid, *Iso-valerianic Acid*, $(CH_3)_2CH.CH_2.COOH$: found as bornyl ester in valerian roots (*V. officinalis* L.) and in free state and as ester in the essential oils of several other plants; the action of the valerian root in medicine in nervous disorders is attributed to this acid or its esters; liquid with smell of putrid cheese; b.p. 175° ; partially soluble in water.

n-Caproic Acid, *Hexoic Acid*, $CH_3.(CH_2)_4.COOH$ or $C_6H_{11}.COOH$: found as glyceride in the fat from goat's milk, whence the name (Lat. *caper-goat*), also in butter, *ghee* and coconut oil as the glyceride caproin; oily liquid with a weak unpleasant odour; b.p. 205° ; sp. gr. 0.9294 at 20° ; immiscible with water.

Oenanthic Acid, Heptolic Acid, $\text{CH}_3(\text{CH}_2)_5\text{COOH}$ or $\text{C}_7\text{H}_{13}\text{COOH}$: occurs in calamus oil; liquid with tallow like odour; m.p. -10° ; b.p. 223.5° ; sp. gr. 0.9183 at 20° .

n-Caprylic Acid, n-Octylic Acid, $\text{CH}_3(\text{CH}_2)_6\text{COOH}$ or $\text{C}_8\text{H}_{17}\text{COOH}$: occurs in the fat from goat's milk; whence the name; in butter, ghee and cocoanut oil as the glyceride caprylin and in various essential oils as ester; liquid m.p. 16° ; b.p. 237.5° ; sp. gr. 0.9139 at 20° ; very sparingly soluble in water; easily soluble in alcohol and ether.

n-Capric Acid, n-Decylic Acid, $\text{CH}_3(\text{CH}_2)_8\text{COOH}$ or $\text{C}_{10}\text{H}_{21}\text{COOH}$: occurs as glyceride in the fat from goat's milk; whence the name, in butter, ghee and cocoanut oil as caprin; solid, m.p. 31.5° ; b.p. 270° ; sp. gr. 0.895 at 30° ; almost insoluble in water, easily soluble in alcohol and ether.

Lauric Acid, Dodecylic Acid, $\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$ or $\text{C}_{12}\text{H}_{25}\text{COOH}$: occurs as ester in cocoanut oil and in various other oils from vegetable sources; crystalline solid, m.p. 44° ; insoluble in water but easily soluble in alcohol and ether.

Myristic Acid, Tetradecylic Acid, $\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$ or $\text{C}_{14}\text{H}_{29}\text{COOH}$: occurs as glyceride in nutmeg oil, cocoanut oil and in other oils of vegetable origin; crystalline solid, m.p. 53.8° ; easily soluble in absolute alcohol, ether, chloroform and benzene.

Palmitic Acid, Hexadecylic Acid, $\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$ or $\text{C}_{16}\text{H}_{33}\text{COOH}$: principal constituent of solid animal fats; occurs also in vegetable oils such as palm oil, etc.; colourless crystalline solid m.p. 62.5° ; insoluble in water; 100 parts of absolute alcohol dissolve 9.32 of acid at 19.5° ; easily soluble in boiling alcohol and in ether but soluble with difficulty in petroleum ether.

Stearic Acid, Octadecylic Acid, $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$ or $\text{C}_{18}\text{H}_{37}\text{COOH}$: occurs as glyceride in solid animal fats and also in vegetable oils; colourless crystalline solid, m.p. 69.5° ; insoluble in water; soluble in 40 parts of cold alcohol and one part of alcohol at 50° .

Arachidic Acid, Eicosane Acid, $\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$ or $\text{C}_{20}\text{H}_{41}\text{COOH}$: occurs as glyceride in notable quantities in groundnut oil (*Arachis hypogaea* L.), whence the name, in butter fat and as an ester or in the free state in various oils; colourless crystalline solid, m.p. 77° ; easily soluble in ether, chloroform, ligroin and benzene but not in absolute alcohol.

Cerotic Acid, $\text{C}_{26}\text{H}_{53}\text{COOH}$: occurs in the free state in bees wax, as an ester in Chinese wax, wool wax, and in opium wax; colourless crystalline solid, m.p. 78° ; soluble in acetone, benzene, ether, chloroform and in boiling alcohol.

Melissic Acid, $\text{C}_{27}\text{H}_{55}\text{COOH}$: occurs in the free state in bees wax; colourless crystalline solid, m.p. 90° ; soluble in chloroform, ligroin and hot alcohol, almost insoluble in ether and methyl alcohol.

Some Halogen Derivatives of Fatty Acids.

Monochloroacetic Acid, $\text{CH}_3\text{Cl.COOH}$; prepared by the action of dry chlorine on glacial acetic acid in presence of red phosphorus, and purified by fractional distillation; crystalline solid, m.p. 63° , b.p. 189° ; the solid or vapour has corrosive action on the skin; easily soluble in water; used in the preparation of synthetic indigo.

Dichloroacetic Acid, CHCl_2COOH ; prepared by the action of potassium ferrocyanide on chloral hydrate; liquid, m.p. 10.8° , b.p. 194.4° ; soluble in water.

Trichloroacetic Acid, CCl_3COOH ; prepared by the oxidation of chloral hydrate with conc. nitric acid; deliquescent colourless crystals with corrosive action on the skin; m.p. 57° , b.p. 196.5° ; soluble in water; used for the treatment of certain skin diseases and as a reagent for testing albumin in the urine and also for precipitating proteins of blood and other tissues in biochemical and toxicological analysis.

Monoiodoacetic Acid, $\text{CH}_3\text{I.COOH}$; the ethyl ester of this acid (see below) is used as tear gas.

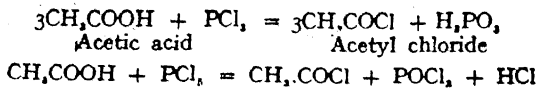
Ethylthiodoacetate, $\text{CH}_3\text{I.COOC}_2\text{H}_5$; prepared by double decomposition of the corresponding chlorine compound (ethyl chloroacetate) with KI in alcoholic solution; colourless oily liquid, sp. gr. 1.8, b.p. 180° ; at 20° its volatility is 3.1 mg. per litre; is extremely irritant and lachrymatory, used in the first Great War (1914-18) as tear gas; lowest lachrymatory concentration is 0.0014 mgm. per litre; intolerable conc. is 0.015 mgm. per litre; a conc. of 1.5 mgm. per litre is toxic on 10 minutes' exposure.

ACID CHLORIDES

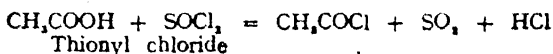
When the OH group of the —COOH radical of an organic acid is replaced by a chlorine atom we get an acid chloride; *e.g.*, acetyl chloride $\text{CH}_3\text{CO.Cl}$ from acetic acid CH_3COOH , propionyl chloride $\text{CH}_3\text{CH}_2\text{CO.Cl}$ from propionic acid $\text{CH}_3\text{CH}_2\text{COOH}$, and so on. The acid chlorides, also known as *acyl chlorides*, thus contain the monovalent radical, —COCl and they all possess properties peculiar to this reactive group.

General Methods of Preparation of Acid Chlorides.

(1) By the action of phosphorus trichloride or pentachloride on an acid: *e.g.*,



(2) By the action of thionyl chloride on an acid: *e.g.*,



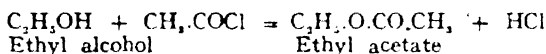
General Properties and Reactions of Acid Chlorides.

These are colourless fuming liquids or solids with a pungent smell. They show great reactivity as will be evident from the following:—

With *water*, an acid chloride is readily converted into the corresponding acid; the fuming in moist air is due to this reaction:

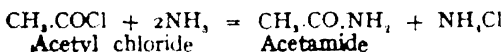


With an *alcohol*, an acid chloride produces an ester, the H of the OH group of an alcohol being replaced by an acyl group: *e.g.*,

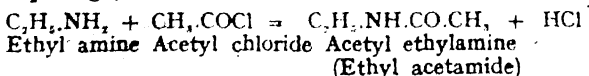


This reaction is utilized not only for the preparation of an acyl derivative for the identification of an organic compound containing OH groups but also for the detection and estimation of OH groups.

With *ammonia*, an acid chloride gives an acid amide, the chlorine atom being replaced by an amino group: *e.g.*,



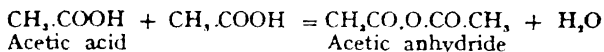
With an *amine*, an acid chloride produces a substituted acid amide, one of the H atoms of the amine being replaced by an acyl group: *e.g.*,



Acetyl Chloride, CH_3COCl : prepared by heating glacial acetic acid with PCl_5 ; colourless pungent liquid which fumes strongly in moist air; b.p. 51° , sp. gr. 1.1051 and 20° ; the reactions of this compound have been discussed in the foregoing equations.

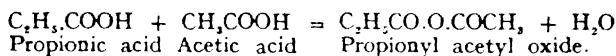
ACID ANHYDRIDES

These are formed by the combination of two molecules of a monobasic acid with the elimination of a molecule of water. These acid anhydrides may also be regarded as *acyl oxides*, i.e. the oxides of two acyl radicals, just as ethers are the oxides of two alkyl radicals. Thus acetic anhydride may be called acetyl oxide $(\text{CH}_3\text{CO})_2\text{O}$, and so on. When the acyl groups are derived from two different acids, instead of one, we get a *mixed anhydride*:



Acetic acid

Acetic anhydride



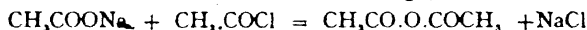
Propionic acid

Acetic acid

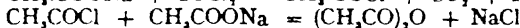
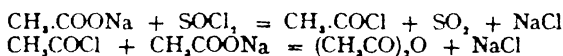
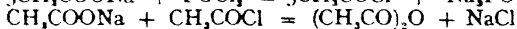
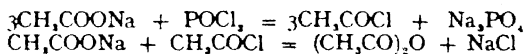
Propionyl acetyl oxide.

General Methods of Preparation of Acid Anhydrides.

(I) By heating a mixture of the *anhydrous* sodium salt of an acid with an acid chloride: *e.g.*,

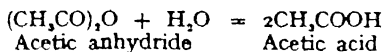


(2) By the action of phosphorus oxychloride or thionyl chloride on *excess* of the anhydrous sodium salt of an acid:
e.g.,



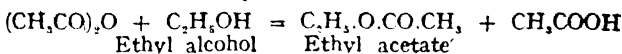
General Properties and Reactions of Acid Anhydrides.

These are neutral colourless liquids or solids with a pungent smell. They do not fume in moist air and act less energetically than the acid chlorides although the reactions are very similar, as the following equations will show:



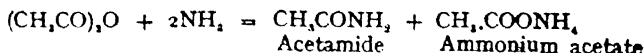
Acetic anhydride

Acetic acid



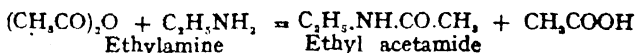
Ethyl alcohol

Ethyl acetate



Acetamide

Ammonium acetate



Ethylamine

Ethyl acetamide

Acetic Anhydride, $(\text{CH}_3\text{CO})_2\text{O}$: prepared by treating fused (*i.e.*, anhydrous) sodium acetate with acetyl chloride and distilling; colourless neutral liquid with a pungent smell; b.p. 136.5° ; sp. gr. 1.0757 at 21° ; like acetyl chloride it is used for the preparation of an acetyl derivative and for estimating the number of alcoholic OH groups in a molecule by introducing the acetyl radical into the molecule, the process being known as *acetylation*; the reactions have been discussed in the above equations. Of the two acetylating agents acetyl chloride and acetic anhydride, the latter is easier to manipulate and cheaper than the other. It is an important reagent used extensively in the manufacture of synthetic chemicals.

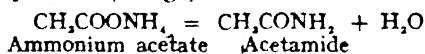
ACID AMIDES.

These may be regarded as acids in which the OH radical of the COOH group is replaced by an amino group; *e.g.*, $\text{CH}_3\text{COOH} \rightarrow \text{CH}_3\text{CO.NH}_2$. They may also be regarded as derivatives of ammonia in which a hydrogen atom is replaced by an acyl group: *e.g.*,

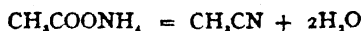


General Methods of Preparation

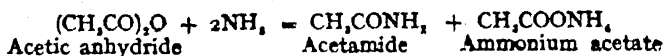
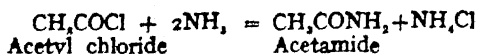
(1) By heating the ammonium salts of fatty acids (partial dehydration): *e.g.*,



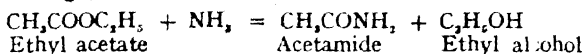
On complete dehydration, *e.g.*, by heating with P_2O_5 , an alkyl cyanide is formed:



(2) By the action of acyl chlorides or acyl anhydrides upon ammonia: *e.g.*,

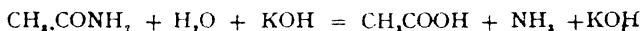
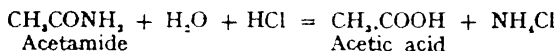


(3) By heating esters with concentrated aqueous ammonia: *e.g.*,

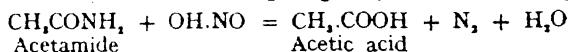


General Properties and Reactions of Acid Amides.

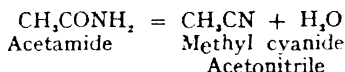
The lowest member, formamide, is a colourless liquid. The higher members are colourless crystalline solids with comparatively high boiling points. The lower members are soluble in water. They are *amphoteric* in nature forming unstable salts like $\text{CH}_3\text{CONH}_2\cdot\text{HCl}$ and $(\text{CH}_3\text{CONH})_2\text{Hg}$. When heated with *dilute acids* or *alkalies* they are hydrolyzed to the corresponding acids: *e.g.*,



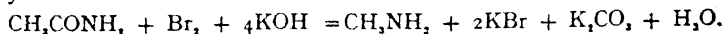
With *nitrous acid*, an acid amide will yield the corresponding acid with evolution of nitrogen gas (*cf.* amines): *e.g.*,



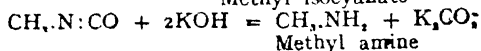
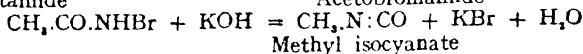
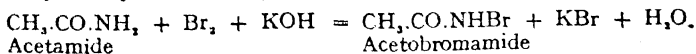
On heating with *dehydrating agents* like phosphorus pentoxide, the corresponding alkyl cyanide or nitrile is produced which on the other hand yields ammonium acetate on complete hydrolysis (see p. 240), *e.g.*,



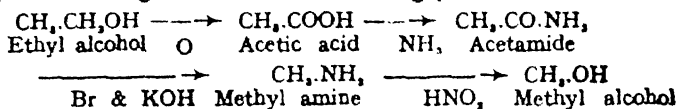
When heated with *bromine and caustic potash*, an acid amide yields an amine:



The reaction takes place in three stages, acetobromamide and methyl isocyanate being formed as intermediate compounds:



This reaction, known as *Hofmann's reaction* or *Hofmann's degradation method*, enables us to pass from a higher homologue to a lower one; e.g.,



Thus, a compound with two atoms of carbon is changed into a compound containing one atom of carbon only.

Formamide, H.CO.NH_2 ; prepared by neutralizing anhydrous formic acid with conc. ammonia, heating the salt in a current of dry ammonia at 100° to 180° and finally in high vacuo; colourless neutral liquid, b.p. $85^\circ\text{--}95^\circ$ (0.5 mm.), $105^\circ\text{--}106^\circ$ (11 mm.); sp. gr. 1.1394, at 20° ; soluble in water and alcohol in all proportions, very slightly soluble in ether; soluble in glycerine; dissolves sugars like glucose.

Acetamide, $\text{CH}_3\text{CO.NH}_2$: Prepared by distilling ammonium acetate and collecting the distillate above 215° ; colourless crystalline solid with a smell of mice, m.p. 82° , b.p. 222° ; easily soluble in water and alcohol; almost insoluble in ether; soluble in glycerine; the reactions of acetamide have been already discussed in the above equations.

CHAPTER XIII

UNSATURATED MONOBASIC FATTY ACIDS AND ESTERS

UNSATURATED MONOBASIC FATTY ACIDS

Fatty acids corresponding to the ethylene, acetylene or other series of unsaturated hydrocarbons are known as unsaturated fatty acids. The higher members of unsaturated fatty acids with one double bond are known to occur in most vegetable oils and in some fats, the higher members with two or three double bonds occur in the vegetable oils known as drying or semi-drying oils (see below) while those with a still higher number of double bonds occur in certain fish oils. A few unsaturated fatty acids with hydroxyl groups are found in vegetable oils and the purgative action of certain oils is attributed to some of these hydroxylated unsaturated acids. Another important series of unsaturated fatty acids, the chaulmoogric acid series, which cannot strictly speaking be included under aliphatic compounds owing to their having a ring structure, should however be discussed under this heading since they possess many of the properties common to fatty acids due to the long straight chain along with the ring structure.

The unsaturated fatty acids would naturally show many of the properties common to unsaturated bonds. Thus, they would combine with gaseous hydrogen in presence of catalysts and yield the corresponding saturated fatty acids (see *hydrogenation*, on p. 168). They decolorize bromine-water and also an alkaline solution of potassium permanganate. They combine with iodine at the double bonds and hence possess *iodine values* (see). As a rule, these unsaturated fatty acids have lower melting points than those of the corresponding saturated fatty acids, as the following figures will show:—hypogæic acid $C_{16}H_{30}O_2$, m.p. 33° and palmitic acid $C_{16}H_{32}O_2$, m.p. 62.5° ; oleic acid $C_{18}H_{34}O_2$, m.p. 14° and stearic acid $C_{18}H_{36}O_2$, m.p. 69.5° ; and so on. The corresponding glycerides also show similar differences. Thus the triglyceride

of oleic acid, *triolein*, is a liquid having a low melting point (-4° to -5°) while the triglyceride of stearic acid, *tristearin* is a solid having a m.p. of 71.5° . This fact shows that oils contain a preponderating amount of glycerides of unsaturated fatty acids.

Classification,

- (1) *The Oleic Acid Series* (with one double bond)
e.g., oleic acid from olive oil, etc.
- (2) *The Chaulmoogric Acid Series* (with one double bond in a five-carbon atom ring and with a long straight chain): e.g., chaulmoogric acid from chaulmoogra oil, etc.
- (3) *The Linolic or Linoleic Acid Series* (with two double bonds) e.g., linolic acid from linseed oil, poppy seed oil, etc.
- (4) *The Linolenic Acid Series* (with three double bonds): e.g., linolenic acid from linseed oil, etc.
- (5) *The Clupanodonic Acid Series* (with four double bonds): e.g., clupanodonic acid from fish oils such as sardine, herring and whale oils, etc.
- (6) *The Ricinoleic Acid Series* (with one double bond and one hydroxyl group): e.g., ricinoleic acid from castor oil.
- (7) *The Acetylinic Acids* (with one triple bond):
e.g., propiolic acid.

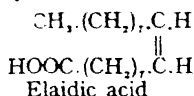
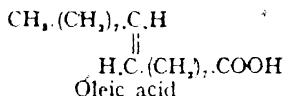
I. The Oleic Acid Series, $C_nH_{2n-2}O_2$

The acids of this series contain two atoms of hydrogen less than the corresponding saturated fatty acids, i.e., they possess one double bond. Hence they would combine with two atoms of hydrogen or with two atoms of bromine or iodine, and so on. The lead salts of the higher members of this series are easily soluble in ether and they are separated by this means from the saturated fatty acids the lead salts of which are insoluble.

(a) **Oleic Acid**, $CH_3.(CH_2)_7.CH:CH.(CH_2)_7.COOH$, $C_{18}H_{34}O_2$. This acid occurs as a triglyceride, known as *olein* or *triolein*, in fair quantities in nearly all vegetable oils (e.g., olive oil, ground nut oil, almond oil, linseed oil, etc.),

in animal oils like cod liver oil and in smaller amounts in body fats of human beings and animals (*e.g.* tallow, etc.). It is easily prepared by saponifying tallow, precipitating the soap solution with lead acetate, drying the lead salt and extracting this with ether, decomposing the lead oleate extracted by ether with HCl. Pure oleic acid is a colourless oily liquid without any taste or smell; m.p. 14° ; b.p. 223° (10 mm.); sp. gr. 0.8998 at 11.8° ; iodine value 90.0; insoluble in water but dissolves in absolute alcohol and ether. In presence of a catalyst such as Ni and at high temperature it combines with 2 atoms of hydrogen (*vide* hydrogenation) and gives stearic acid.

The solubility of the dry lead salt in ether is utilized in separating oleic acid from palmitic, stearic and other solid fatty acids. Oleic acid penetrates the skin more readily than fats and oils and is, therefore, used for compounding medicines, such as mercuric oxide, for external application. It is also used in the preparation of soaps for delicate fabrics. When treated with nitrous acid, oleic acid is converted into its solid stereoisomer (geometrical isomer) known as *elaidic acid* (m.p. 44.5°); this serves to identify oleic acid.

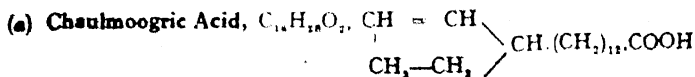


(b) **Erucic Acid**, $\text{C}_{22}\text{H}_{42}\text{O}_2$, $\text{CH}_3(\text{CH}_2)_7\text{CH}:\text{CH}(\text{CH}_2)_{11}\text{COOH}$

This occurs as a glyceride in the oil from the seeds of white and black mustard, in rape oil and in other oils; colourless crystalline substance, m.p. 34° ; insoluble in water, easily soluble in alcohol and ether.

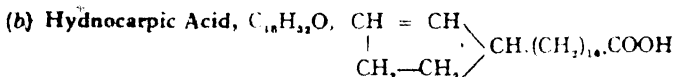
II. The Chaulmoogric Acid Series, $\text{C}_{11}\text{H}_{20-4}\text{O}_2$

The acids of this series contain a five-carbon atom ring with a double bond, and a long straight chain. Having an asymmetric carbon atom (in the ring), these acids show optical activity. They have a special medicinal value since the soluble salts and the esters are widely used in the *treatment of leprosy*. The chaulmoogric, hydnocarpic and other acids of this homologous series occur as glycerides in the fatty oils, known as *chaulmoogra oils*, obtained from the seeds of several species of *Hydnocarpus*, *e.g.*, *H. wightiana* Bl., *H. anthelmintica* Pierr., *H. venenata* Gærtn., *H. kurzii* Wrbg. (*Taraktogenos kurzii* King), etc., found in different parts of India, Siam, Philippines, and other places.



It crystallizes from petroleum ether or alcohol in colourless glistening leaflets, easily soluble in ether and chloroform but soluble with difficulty in other organic solvents; m.p. 68° ; b.p. 248° (20 mm.); iodine value 90.6; sp. rotation $[\alpha]_D = +56^\circ$ (in chloroform).

The ethyl ester of this acid, known as *ethyl chaulmoograte*, $C_{11}H_{21} \cdot \text{COOC}_2\text{H}_5$, is a colourless liquid; b.p. 230° (20 mm.); sp. gr. 0.9064 at 15° ; sp. rotation $[\alpha]_D^{20} = +50.7^\circ$ (pure liquid).



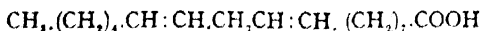
It crystallizes from hot alcohol or ethyl acetate in colourless lustrous leaflets, soluble in ether and chloroform but with difficulty in other organic solvents; m.p. 60° ; iodine value 100.7; sp. rotation $[\alpha]_D = +68.1^\circ$ (in chloroform).

Ethyl hydnocarpate, $C_{18}H_{32} \cdot \text{COOC}_2\text{H}_5$, is a colourless liquid having a sp. rot. $[\alpha]_D = +51.6^\circ$ (in chloroform); b.p. 211° (19 mm.).

III. Linolic or Linoleic Acid Series, $C_nH_{2n-4}O_2$.

The acids of this series contain two double bonds *i.e.*, four hydrogen atoms less than the corresponding saturated acids, and hence form tetrabromides by which they are identified. Glycerides of these acids and those of the linolenic acid series occur in *drying oils* or *semi-drying oils*, such as linseed oil, sesame oil, poppy seed oil, etc. The name '*drying*' is derived from the fact that when exposed to the air in thin layers, the oil absorbs oxygen from the air and is changed into a transparent solid resinous mass. These oils are, therefore, utilized in making oil-cloth, linoleum and various paints and varnishes. The lead salts are easily soluble in ether. The barium salts are soluble in ether containing a small proportion of alcohol.

(a) **Linoleic or Linolic Acid**, $C_{18}H_{32}O_2$



This occurs as a glyceride in drying and semi-drying oils such as poppy seed oil, linseed oil, sesame oil, cotton seed oil, etc. It is colourless oily liquid, soluble in alcohol and ether; sp. gr. 0.9026 at 18° ; b.p. 228° (14 mm.); iodine value 181.3; on hydrogenation, it combines with 4 atoms of hydrogen to give stearic acid; yields a tetrabromide $C_{18}H_{32}O_2Br_4$, m.p. 114° .

IV. The Linolenic Acid Series, $C_nH_{2n-6}O_2$

The acids of this series occur as glycerides in drying and semi-drying oils such as linseed oil, poppy seed oil, cotton seed oil, etc., and contain 6 atoms of hydrogen less than the corresponding saturated fatty acids. They possess three double bonds and decolorize bromine water and alkaline permanganate readily. The formation of crystalline tetra- and hexabromides with Br serves as a test for their identification and is utilized in detecting linseed oil in edible oils. Their lead salts and barium salts are easily soluble in ether.

To carry out the *hexabromide test* for linseed oil, 0.5 c.c. of the oil is taken in a dry test tube and to this are added 10 c.c. of the bromine reagent (Br 1 part, nitrobenzene 4 parts, glacial acetic acid 28 parts) and the test tube is closed and shaken. A yellow precipitate is at once formed and settles rapidly which is not soluble in ether.

(a) **Linolenic Acid**, $C_{11}H_{18}O_2$,



This acid occurs as a glyceride in linseed oil, poppy seed oil, etc.; colourless oily liquid; hexabromide, m.p. 180° . On hydrogenation it gives stearic acid.

V. The Clupanodonic Acid Series, $C_nH_{2n-8}O_2$

The acids of this series contain 8 hydrogen atoms less than the corresponding saturated fatty acids. They are known to occur as glycerides in fish oils such as Japanese sardine oil, herring oil, whale oil, etc. The fishy smell of these oils is due to these acids and some of their oxidation products.

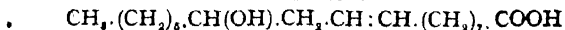
(a) **Clupanodonic Acid**, $C_{19}H_{28}O_2$

A pale yellow liquid with fishy smell; octabromide $C_{19}H_{28}O_2Br_8$ blackens at 200° and decomposes without melting; on hydrogenation it gives stearic acid.

VI. The Ricinoleic Acid Series, $C_nH_{2n-2}O_3$

The acids of this series contain one double bond and a hydroxyl group. They are optically active due to an asymmetric C atom and yield acetyl derivatives due to OH group.

(a) **Ricinoleic Acid**, $C_{18}H_{34}O_3$,



It may be considered as a hydroxy derivative of oleic acid. As glyceride it occurs as the chief constituent of castor oil; oily liquid, m.p. 4° — 5°C ; easily soluble in alcohol and ether; it shows the sp. rotation $[\alpha]_D = +6.67^{\circ}$ (pure liquid); $[\alpha]_D = +6.25^{\circ}$ to $+7.5^{\circ}$ (in acetone).

VII. The Acetylenic Acid Series, $\text{C}_n\text{H}_{2n-4}\text{O}_2$

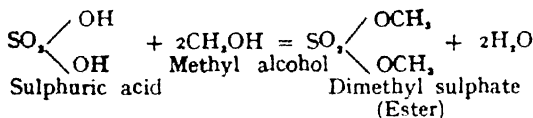
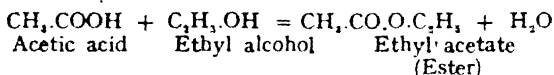
These contain one triple bond and are prepared synthetically.

(a) Propargylic or Propiolic Acid, $\text{CH} \equiv \text{C} \cdot \text{COOH}$

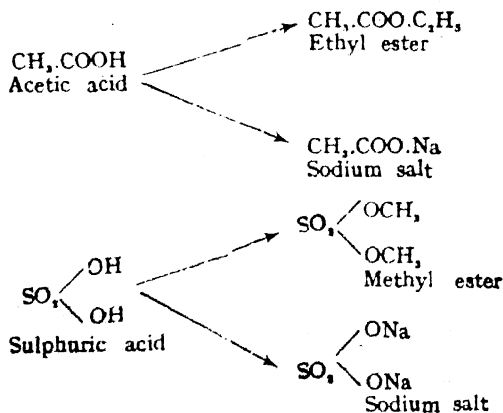
Liquid; boils with decomposition at 144° ; smells like acetic acid; soluble in water, alcohol, ether and chloroform; yields explosive compounds with ammoniacal cuprous chloride or ammoniacal silver nitrate (*vide acetylene*, p. 63).

ESTERS.

Organic and inorganic acids combine with alcohols with the elimination of water and form esters: thus,

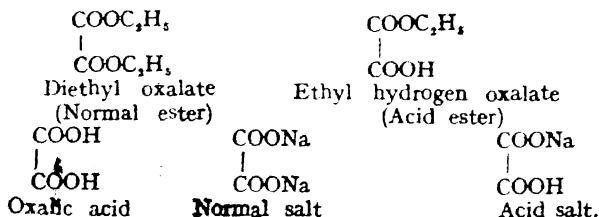


We may thus *define* esters as derivatives of acids formed by the replacement of the acidic hydrogen atoms of the acids by alkyl groups. The reaction is similar to the formation of a salt by the replacement of the acidic hydrogen atom by a metal: *e.g.*,

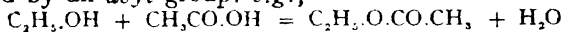


There is, however, a difference between an ester and a salt in that whereas the metal in a salt will *ionize* in solution, the alkyl group will never ionize.

In the case of dibasic acids, we may get either *normal* or *acid* esters just as we get either normal or acid salts: *e.g.*,



We may also regard esters as derivatives of alcohols in which the hydrogen atom of the *alcoholic OH* group is replaced by an *acyl* group: *e.g.*,



It is, however, more convenient to remember esters as derivatives of acids as discussed above. Formerly, esters used to be known as ethers and that is why the ester ethyl acetate is still called acetic ether.

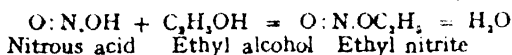
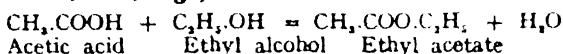
Occurrence of Esters.

Many of the esters have a fragrant odour and the sweet smell of flowers, fruits and other parts of plants are due to the presence of esters. In the *essential oils* obtained from plants, the esters often form the main components. Many esters are prepared synthetically for making artificial *flower* and *fruit essences* and *perfumes*. Thus amyl acetate has the smell of banana, methyl butyrate has the smell of pine-apples, isoamyl isovalerate has the smell of apples, and so on, and these are thus prepared synthetically as artificial flavouring agents. Some esters, such as ethyl acetate, amyl acetate, butyl acetate, glycol diacetate, etc., are used as *solvents* for industrial purposes. Esters such as methyl salicylate, ethyl nitrite, amyl nitrite, etc., are used in *medicine*. The naturally occurring vegetable or animal *oils and fats*, which form important articles of our *food*, are all esters of the trihydric alcohol glycerol with various fatty acids. The *waxes* are esters of higher monohydric alcohols and higher fatty acids,

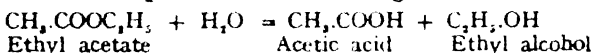
and the *lipines* (or lecithins) found in our body tissues are esters of glycerol with fatty acids and phosphoric acid.

General Methods of Preparation of Esters.

(1) By heating an acid and an alcohol in presence of dehydrating agents such as conc. sulphuric acid, anhydrous zinc chloride, etc.; *e.g.*,

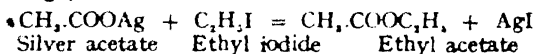


The process of the conversion of an acid into an ester is known as *esterification*. The reaction is a *reversible* one (see under Ethyl Acetate), since the water formed reacts with the ester with the regeneration of the free acid and alcohol, and the object of the dehydrating agent used for the esterification is to prevent the following reverse reaction:

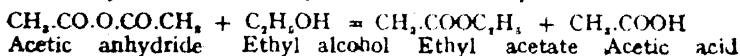
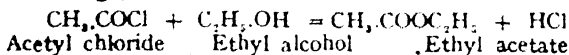


This reverse process, *i.e.*, the decomposition of an ester into an acid and an alcohol with the help of water is called *hydrolysis*.

(2) By heating the silver salt of an acid with an alkyl iodide: *e.g.*,



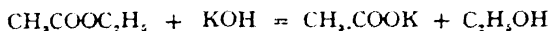
(3) By heating acid chlorides or acid anhydrides with alcohols: *e.g.*,



General Properties and Reactions of Esters.

The normal esters are neutral in reaction, almost insoluble in water but soluble in ether, chloroform or absolute alcohol. Most of them are colourless liquids lighter than water and many possess a pleasant smell.

They are slowly hydrolyzed by water and the hydrolysis occurs more readily when heated with dilute acids or alkalies. In the case of esters which are not easily hydrolyzed by heating with aqueous alkalies, they are more readily hydrolyzed by heating with an *alcoholic* solution of *caustic potash* in which the esters are more easily soluble:



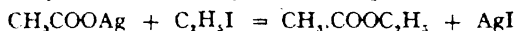
The hydrolysis of an ester with the help of an alkali, like NaOH or KOH, is sometimes spoken of as *saponification*, the process being similar to the conversion of a fat or oil into an alcohol and the Na or K salts of higher fatty acids which are used as *soaps* (see p. 168). *Hydrolysis* is, however, a general term meaning the decomposition of a larger molecule into simpler components with the help of water, and we will come across several examples of hydrolysis amongst carbohydrates, proteins, etc.

Esters of Acetic Acid,

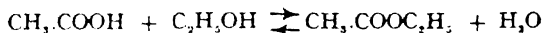
Methyl Acetate, $\text{CH}_3\text{COOCH}_3$, : prepared by distilling acetic acid and methyl alcohol in presence of some conc. sulphuric acid; colourless liquid with a pleasant ethereal smell; soluble in three parts of water at 22° ; easily soluble in ether, chloroform or absolute alcohol; b.p. 57.5° (760 mm.); sp. gr. 0.9280 at 20° ; used as a solvent for celluloid and as an artificial fruit essence.

Ethyl Acetate, Acetic Ester, Acetic Ether, $\text{CH}_3\text{COOC}_2\text{H}_5$:

(1) By action of ethyl iodide upon silver acetate:



(2) By heating glacial acetic acid with absolute alcohol:



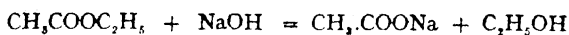
This reaction is a *reversible* one, since the water formed reacts with the ester with the regeneration of the free acid and the alcohol, and finally a *dynamic equilibrium* is established between the forward and backward reactions. This forms a typical example of the truth of the *Law of Mass Action* and it has been found by actual experiment that when equilibrium is established, about 67 per cent of the acid is esterified. To help the forward reaction, one may do it either by a large increase in the concentration of the alcohol or by a decrease

of the backward reaction by the use of a dehydrating agent to remove the water formed. The latter is found to be more economical and hence the dehydrating agent conc. sulphuric acid is used in its preparation.

In the laboratory, ethyl acetate is best prepared in the following manner: A mixture of 50 c.c. of conc. sulphuric acid and 50 c.c. of alcohol is taken in a distilling flask which is fitted with a tap funnel containing a mixture of 50 c.c. of alcohol and 50 c.c. of glacial acetic acid. The flask is heated over a sand bath at 140° and the mixture of alcohol and acetic acid is allowed to flow in at the same rate as the ethyl acetate distils over. The distillate which may contain along with the ethyl acetate a mixture of alcohol, acetic acid, ether, sulphur dioxide and water, is taken in a separating funnel and washed first with a dilute solution of sodium carbonate to remove the free acids and then with a 50 per cent solution of CaCl_2 to remove alcohol. The ethyl acetate is next dried with anhydrous CaCl_2 and distilled.

Properties, Uses and Reactions of Ethyl Acetate.

It is a colourless liquid with a pleasant smell and is neutral in reaction. It dissolves in water to the extent of about 7.9 per cent at 15° , and 28 parts of ethyl acetate dissolve one part of water. It dissolves easily in ether, chloroform or absolute alcohol. It boils at 77.1° . (760-mm.) and has a sp. gr. of 0.8990 at 20° . It is used as a solvent in the laboratory as well as in industry. It is also used as a fruit essence. It is hydrolyzed by dilute alkalies to ethyl alcohol and the alkali salt of acetic acid:



Halogen derivatives of ethyl acetate such as ethyl chloroacetate $\text{CH}_2\text{Cl.COOC}_2\text{H}_5$, ethyl bromo acetate, and ethyl iodoacetate were used in the first Great War as tear gases (see p. 145).

Esters of Nitrous Acid and Nitric Acid.

Ethyl Nitrite, $\text{C}_2\text{H}_5\text{O.NO}$; prepared by treating conc. nitric acid with a cold mixture of alcohol and conc. sulphuric acid in presence of copper turnings and subsequent distillation; colourless liquid with pleasant smell; b.p. 17° ; almost insoluble in water, soluble in alcohol; an alcoholic solution is used in medicine under the name of

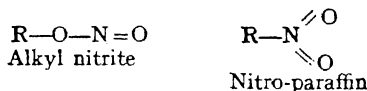
aetheris nitrosi (sweet spirit of nitre) as a stimulant, antispasmodic and carminative.

Ethyl Nitrate, $C_2H_5O.NO_2$: prepared by distilling a mixture of $AgNO_3$ and C_2H_5I : $AgNO_3 + C_2H_5I = AgI + C_2H_5O.NO_2$. It is a colourless liquid with a fragrant smell; sparingly soluble in water; b.p. 87° .

Amyl Nitrite, $C_5H_{11}O.NO$: the amyl nitrite used in medicine is made from commercial amyl alcohol which contains about 87 per cent of isoamyl alcohol $(CH_3)_2CH.CH_2.CH_2OH$, the remainder being chiefly active amyl alcohol $CH_3.CH_2.CH(CH_3).CH_2OH$, and thus the nitrite produced is really a mixture of the nitrites of the above two amyl alcohols. Amyl nitrite is prepared by adding conc. sulphuric acid drop by drop to a well cooled mixture of amyl alcohol and powdered sodium nitrite. The mixture is then poured into cold water and the amyl nitrite separated, washed with a little water, dried with anhydrous $CaCl_2$ and distilled. The B. P. amyl nitrite is a clear yellow liquid with a fragrant odour and a pungent taste, b.p. $90^\circ-100^\circ$. Pure isoamyl nitrite is a clear yellow liquid with a fragrant odour, soluble with difficulty in water but easily soluble in alcohol and ether; b.p. 98° , sp. gr. 0.8717 at 20° . Amyl nitrite on inhalation produces dilatation of blood vessels and relaxation of spasms and is, therefore, used as a valuable medicine in cardiac pain (angina pectoris) and in bronchial asthma.

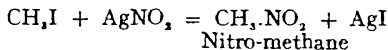
Nitro Paraffins.

These form a class of compounds isomeric with alkyl nitrites as the following structural formulae will show:



Preparation of Nitro-paraffins.

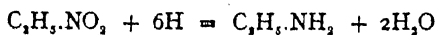
The lower members of the nitro-paraffins are obtained by distilling an alkyl iodide with solid silver nitrite: thus,



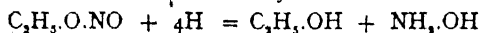
The higher members, from heptane upwards, can be obtained by direct nitration of the hydrocarbons with fuming nitric acid. This direct nitration is fairly common amongst compounds of the aromatic series.

Properties and Reactions of Nitro-paraffins.

The lower members are pleasant smelling liquids having higher boiling points than the isomeric nitrites (e.g. nitroethane b.p. 114° , ethyl nitrite b.p. 16°). When reduced, the nitro paraffins yield primary amines whereas the alkyl nitrites yield the corresponding alcohol and hydroxylamine: e.g.,

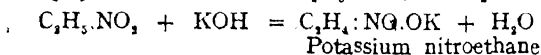
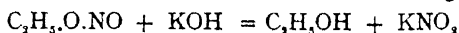


Nitro ethane Ethyl amine



Ethyl nitrite Ethyl alcohol Hydroxylamine

Alkyl nitrites are easily hydrolyzed by caustic alkalies whereas the nitro paraffins behave as acids and form salts: *e.g.*,



Potassium nitroethane

Nitro-ethane, $\text{C}_2\text{H}_5\cdot\text{NO}_2$: prepared by the action of silver nitrite on ethyl iodide; liquid, insoluble in water; b.p. 114° .

Esters of Sulphuric Acid.

Dimethyl Sulphate, $\text{CH}_3\text{O}\cdot\text{SO}_2\cdot\text{OCH}_3$: prepared by distilling methyl alcohol with conc. sulphuric acid; colourless poisonous liquid; b.p. 188° ; used as a methylating agent in synthetic preparations.

Diethyl Sulphate, $\text{C}_2\text{H}_5\text{O}\cdot\text{SO}_2\cdot\text{OC}_2\text{H}_5$: prepared by the action of ethyl iodide on silver sulphate; colourless liquid with an odour of peppermint; insoluble in water and neutral in reaction; b.p. 96° (15 mm.).

Ethyl Hydrogen Sulphate, Sulphovinic acid $\text{C}_2\text{H}_5\text{O}\cdot\text{SO}_2\cdot\text{OH}$; prepared by heating alcohol with conc. sulphuric acid, or by passing ethylene into fuming sulphuric acid; colourless syrupy liquid, acid in reaction; easily soluble in water; forms salts, the acidic hydrogen being replaced by a metal.

Esters of Halogen Acids, *e.g.*, Ethyl chloride $\text{C}_2\text{H}_5\text{Cl}$
(see p. 70).

CHAPTER XIV

FATS, OILS, WAXES, STEROLS AND PHOSPHATIDES

Fats and Oils

Nature and Occurrence.

There is no essential difference between a fat and an oil; chemically, both are esters of glycerol with higher fatty acids, but it is customary to designate the liquid members (*i.e.*, those that are liquid at 20°C) as *oils* or *fatty oils* and the solid members (*i.e.*, those that are solid at 20°C) as *fats*. The fatty oils are sometimes called *fixed oils* in order to differentiate them from the essential oils and mineral oils which are volatile. The fixed oils thus form a translucent spot (grease-spot) on paper which cannot be removed by washing with water and subsequent drying. The fats and oils may be of animal or of vegetable origin; some examples of common *animal* fats and oils are butter (from milk), lard (the body fat of hogs), mutton fat, beef fat, neat's foot oil (from the feet of oxen; *neat*—cattle), cod liver oil, shark oil, etc., and examples of some common *vegetable* fats and oils are cacao butter (from the beans of *Theobroma cacao* L.), Chinese vegetable tallow (from the seeds of *Sapium sebiferum* Roxb.), mustard oil, cocoanut oil, ground nut oil, castor oil, olive oil, linseed oil, etc.

Isolation of Fats and Oils.

For animal fats, the tissues are chopped up, the fat melted with the help of steam or boiling water and the molten fat either skimmed off or filtered from the tissues. Cod liver oil is obtained from fresh livers of the cod fish, *Gadus morrhua* (hence, *oleum morrhuae*), by applying low pressure steam at a temperature not exceeding 85°, cooling to 0° and filtering from the separated fat. The vegetable oils and fats present in the seeds are usually obtained by packing them in linen bags and submitting them to high pressure in

a hydraulic press with or without the application of heat (usually steam) according as *hot-pressed* or *cold-drawn* oil is desired. The oil is then refined by filtering through substances such as fuller's earth, animal charcoal, etc., which remove most of the undesirable odour or colour. The oils are also extracted by suitable solvents such as light petroleum, carbon disulphide, carbon tetrachloride, dichloroethylene, etc., and the solvent recovered by distillation. The yield of oil by extraction is higher than by pressing but the cost of extraction is also heavier.

Use of Fats and Oils.

Fats and oils constitute one of the three chief articles (carbohydrates, fats and proteins) of our *diet*. Animals synthesize fats from carbohydrate foods and it is not, therefore, necessary for them to take fatty foods for storing fats in their body. Both in animal tissues and in seeds, the fats and oils act as *reserve fuel or food*. Fats and oils are used as *lubricants* and as *illuminating agents* and in large amounts for the manufacture of various kinds of *soap* and of *glycerol* and *nitroglycerine* and also in the manufacture of *stearin candles*. The drying and semi-drying oils are used in the preparation of *paints and varnishes*.

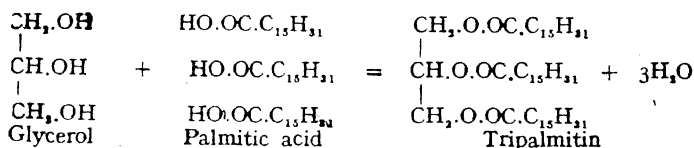
Physical Properties of Fats and Oils.

The physical distinction between fats and oils as solid and liquid members has been mentioned above. There is, however, no sharp line of demarcation between fats and oils and this *physical difference* is chiefly due to a difference in the constituent glycerides, the oils having a larger proportion of the low-melting glycerides. When pure, the oils and fats are colourless, odourless and tasteless, and the so-called characteristic odour or taste is due to the presence of traces of foreign matter. They have a lower *specific gravity* than water (usually varying from 0.910 to 0.970 at 15.5°C) and hence they float on water. The fatty oils are *not volatile with steam* like the essential oils. They produce a permanent translucent spot (grease spot) on paper as mentioned above. They are *insoluble in water* and with the exception of castor oil they *dissolve very sparingly in cold alcohol*. They are dissolved by boiling alcohol to some extent but separate out

again on cooling. They dissolve easily in ether, chloroform, benzene, carbon disulphide, carbon tetrachloride, trichloroethylene, etc., and with the exception of castor oil, they also dissolve in petroleum ether and in paraffin oils.

Chemical Properties and Reactions of Fats and Oils.

The natural fats and oils are all neutral esters of the trihydric alcohol glycerol with the *higher fatty acids* (usually with an *even* number of carbon atoms, such as stearic acid, palmitic acid, etc., each of the hydrogen atoms of the OH groups of glycerol being replaced by an acid radical with the elimination of water. Thus, *tripalmitin* would be formed as follows:—



If all the three replaceable hydrogen atoms of glycerol are replaced by the same acid radical, the glyceride is known as a *pure glyceride*, while if the acid radicals are different the glyceride is called a *mixed glyceride*. These glycerides are designated according to the nature of the fatty acids present. Thus, the ester of glycerol with 3 molecules of stearic acid is called *tristearin* (or simply, *stearin*), the ester with 3 molecules of oleic acid is called *triolein* (or simply, *olein*), these being pure glycerides. Again, the ester of glycerol with 2 molecules of oleic acid and one molecule of palmitic acid is called *palmitodiolein*, the ester with one molecule of oleic acid and 2 molecules of stearic acid is called *oleodistearin*, and the ester with equimolecular proportions of oleic, palmitic and butyric acids is called *oleopalmito butyryn*, these being mixed glycerides. In natural fats and oils, both pure and mixed glycerides are present although there is generally a preponderance of the latter. Thus cacao butter consists mainly of oleopalmitostearin, oleodistearin and steardiolein together with smaller quantities of other glycerides, and olive oil consists mainly of triolein with smaller proportions of other pure and mixed glycerides. The pure glycerides of lower fatty acids such as tributyrin, tri-

Many of the fats and oils contain unsaturated acids such as oleic acid, linolic acid, etc. They absorb iodine at the double bonds and hence the degree of unsaturation is ascertained by the amount of iodine taken up as determined by the iodine values of these oils and fats. The *iodine value* of an oil or a fat is defined as the number of grams of iodine absorbed by 100 grams of the oil or fat under definite conditions of experiment. It is determined by dissolving an accurately weighed amount of the oil or fat (0.2 to 0.5 gram) in 10 c.c. of pure carbon tetrachloride in a glass-stoppered bottle, adding 25 c.c. of the specially prepared solution of iodine (for instance, iodine trichloride in glacial acetic acid) and leaving it in the dark for about half an hour. Some KI solution and water are added and the amount of unabsorbed iodine is ascertained by titration with a standard solution of sodium thiosulphate. The iodine value serves not only for the identification of an oil or fat but also for the detection of its adulteration. Thus, ghee has an iodine value between 26 and 38 and if it is adulterated with an oil like cocoanut oil (I.V. 8-10), the I.V. will be lowered, or if adulterated with groundnut oil (I.V. 85-100), the I.V. will be raised considerably.

The iodine value is also an important means of determining the nature of an oil, and a rough classification into *drying oils* (I.V. above 120; e.g., linseed oil I.V. 175-200), *semi-drying oils* (I.V. 100-120; e.g., cotton seed oil I.V. 105-115) and *non-drying oils* (I.V. below 100; e.g., olive oil I.V. 80-90) is based upon the iodine value.

Besides the *glycerides*, the oils and fats contain small amounts of *non-saponifiable matter* which may be isolated by saponifying the oil or fat with an alkali and extracting an aqueous solution of the saponified product with ether. The non-saponifiable matter consists of substances such as *sterols*, *higher alcohols*, *hydrocarbons*, *vitamins*, *phosphatides*, *ketones*, etc.

Fats and oils are *detected* in animal or vegetable tissues in histological sections by the black colour observed by adding a 1 per cent solution of *osmic acid*.

Hydrogenation of Oils and Fats.

The unsaturated acids and their glycerides are generally more fluid than the corresponding saturated compounds. Cheaper grades of oils and fats are, therefore, made more solid, or *hardened* as it is called, by saturating them with hydrogen, the hydrogen atoms being absorbed at the double bonds. A current of pure hydrogen, prepared electrolytically is passed at a pressure of about 4 atmospheres through the oil or fat heated to about 180° and containing about 1 per cent of freshly reduced finely divided *nickel* which acts as a catalyst. This process is known as *hydrogenation* and it not only reduces the iodine value but also raises the melting point and solidifying point of the oil or fat and removes much of its objectionable odour and taste. It thus raises the value of the cheaper grades of oils and fats and makes them better fitted for the preparation of soap, stearin candles, margarine, etc. The so-called *vegetable ghee* of the Indian market is chiefly obtained by the hydrogenation of oils such as cotton seed oil, ground-nut oil, cocoanut oil, etc.

Soaps.

Fat or oil or a mixture of both is treated with the calculated quantity of NaOH solution in large iron pans and heated by steam until hydrolysis is complete. Common salt is now added and the sodium salts of the fatty acids (*i.e.*, soap) which are less soluble in brine are 'salted out' and float on the surface. The lower aqueous layer, called the 'spent lye', is drawn off and used for the manufacture of glycerol (see). The hot soap is then boiled with water and allowed to cool and solidify. The solid mass is cut up into chips, dried to some extent, mixed with the colouring matter and perfume desired and moulded into shape.

The ordinary toilet soaps are *hard soaps*, *i.e.* the sodium salts of fatty acids while the *soft soaps* are the potassium salts of the fatty acids with some glycerol. *Transparent soaps* are made either by dissolving soap shavings in alcohol or by using alcoholic alkali for hydrolysis; most of the alcohol is distilled off and the gelatinous residue made into cakes. Cheaper grades of soap such as *washing soap* contain *filling materials* such as China clay (kaolin, a natural silicate of aluminium), soapstone (magnesium silicate), fuller's earth (an impure variety of kaolin), etc., and various *medicated soaps* such as sulphur-, creosote-, carbolic-soap, etc., are made by incor-

porating these ingredients with the soap. *Soap powders* are obtained by mixing sodium carbonate with ordinary soaps. *Liquid soaps* are usually potash soaps diluted with water or alcohol and containing some glycerol. The *plaster of lead* of B.P. is a lead soap (mainly lead-oleate), made by boiling olive oil with litharge (PbO).

The *cleansing action of a soap* is due to slow hydrolysis of the soap, which is the salt of a very weak acid, in the presence of water into the free fatty acid and free alkali. Thus,



The free fatty acid forms an *acid salt* with the unhydrolyzed soap and this acid salt gives an opalescent solution and forms the lather by lowering the surface tension of the water. The alkali loosens the dirt which is enveloped and removed by the lather formed. The advantage of a soap over NaOH is that the alkali cannot get concentrated (the soap being regenerated by the reverse action) and thus the skin or the material to be cleaned is protected from the caustic action of the strong alkali.

Water containing soluble calcium or magnesium salts is called *hard water*. On washing with soap the insoluble calcium or magnesium salts of the fatty acids, *i.e.*, Ca or Mg soaps are formed and precipitated and thus some of the soap is wasted before the formation of a lather.

Stearine.

Chemically, 'stearin' should mean, as stated before, the triglyceride of stearic acid, but the *stearine* of commerce used for making stearine candle consists mainly of a mixture of stearic and palmitic acids. It is obtained by hydrolyzing fats or oils, liberating the fatty acids, and cooling and squeezing out the liquid fatty acids by pressing. The hard mass thus obtained is known as *stearine* and it is used not only in the preparation of candles after admixture with some paraffin but also in the manufacture of various toilet preparations.

BUTTER

The fat obtained from cow or buffalo milk known as butter, has the following approximate composition: fat 82 per cent, water 16 per cent, and casein, lactose, inorganic matter, etc., 2 per cent. Butter fat consists of the glycerides of higher fatty acids such as stearic acid, oleic acid, palmitic acid, myristic acid, lauric acid, &c. (together about 91 per cent) and glycerides of lower fatty acids such as capric acid, caprylic acid, caproic acid and butyric acid (together about

9 per cent). Amongst the lower fatty acids, *butyric acid* constitutes nearly half the amount. The high percentage of the glycerides of the lower fatty acids, the *soluble volatile fatty acids*, is characteristic of butter and serves to distinguish butter fat from all other fats. The *Reichert-Meissl* or *Reichert-Wollny value* of a fat depends upon this factor and is defined as the volume (in c.c.) of N/10 KOH required to neutralize the soluble volatile fatty acids obtained from 5 grams of a fat. The Reichert-Meissl value of butter fat or ghee lies between 24 and 40 or more (the maximum being found only in buffalo ghee), whereas in the case of body fats of cows, buffalo and other animals or in vegetable fats and oils it is quite low (*e.g.*, cocoanut oil 6.5—8; other vegetable oils 0.0—0.8; beef-fat 0.3—0.5; and lard 0.2).

Ghee is clarified cow or buffalo butter from which curd and moisture have been removed by melting and straining. The standards laid down in Bengal for Reichert-Meissl values for ghee are: cow, not less than 24; buffalo, not less than 30.

MARGARINE

This is an artificial butter or butter substitute consisting of oils from various sources such as cotton seed oil, cocoanut oil, ground nut oil, etc., mixed with solid fatty acids such as *stearine*, and sterilized skim milk which has been inoculated with the butyric ferment (lactic acid bacilli), carefully grown in milk, so as to impart the blended mixture a butter like odour. Its melting point lies between 22°-27°C.

In some cases animal fats such as beef fat and lard are mixed with the vegetable oils in such a proportion that the melting point lies within the range. Instead of adding animal fats stearine, which is more solid at the ordinary temperature, is sometimes added to the oils to give the desired melting point to the mixture. Hydrogenated fats, *i.e.*, cotton seed and other vegetable oils or whale oil rendered solid by hydrogenation, are now extensively used to replace the animal fats or stearine to adjust the desired consistency. A harmless colouring matter and a little common salt are added finally. Some manufacturers add to it vitamins A and D to render it more nearly equivalent to real butter.

As all the ingredients are refined and sterilized, the final product is perfectly wholesome and its food value in calories is higher than that of butter.

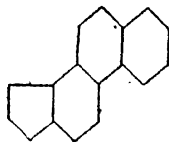
Waxes.

These are the esters of higher fatty acids and higher monohydric alcohols. Thus, myricyl palmitate $C_{15}H_{31}.COO.C_{30}H_{61}$, the ester of palmitic acid and myricyl alcohol $C_{30}H_{61}.OH$, is an important constituent of *bees wax*; ceryl cerotate, the ester of cerotic acid $C_{25}H_{51}.COOH$ and ceryl alcohol $C_{26}H_{53}.OH$ is the main constituent of *Chinese wax* and cetyl palmitate, the ester of palmitic acid with cetyl alcohol $C_{16}H_{33}.OH$ is the chief constituent of *spermaceti*, the wax found in the head of the sperm whale and so on.

Lanolin, Wool wax, Wool fat.—It is the natural greasy substance extracted from sheep's wool and consists of esters of cholesterol (see below) and other sterols with higher fatty acids like oleic acid, myristic acid, carnaubic acid, etc., together with some free cholesterol and other sterols. It absorbs about 50 per cent of water and does not easily get rancid. It is readily absorbed by the skin and thus helps the absorption of drugs through this route and it is, therefore, used in making ointments. It yields on hydrolysis a mixture of fatty acids with cholesterol and other complex alcohols of unknown constitution.

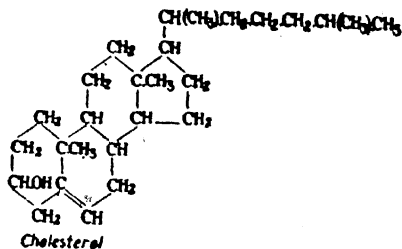
Sterols.

These form a group of monohydric secondary alcohols, usually unsaturated, having a complex ring structure, all possessing the cyclopentenophenanthrene skeleton (see Fig.). They are the normal constituents of oils and fats, both in the animal and vegetable kingdom. As mentioned before, they are isolated from the non-saponifiable fraction of oils and fats. According to the source of the sterols they are sometime classified into (1) *Zoosterols*, i.e., sterols found in animal tissues, e.g., cholesterol, (2) *Phyto-sterols*, i.e., sterols found in vegetable tissues, e.g., sitosterol, stigmasterol, etc., and (3) *Mycosterols*, i.e., sterols



found in fungi, yeast, etc., e.g. ergosterol. They are all crystalline solids (*stereos*—solid; *sterol*—solid alcohol).

Cholesterol, $C_{27}H_{46}.OH$



This is an unsaturated secondary alcohol having a complex ring structure. It occurs in bile, bone marrow, nerve tissues, brain matter, blood corpuscles, blood serum and in various other animal tissues, in egg-yolk, in butter, in cod-liver oil, in wool fat (lanolin), etc. Gallstones consist mainly of cholesterol and hence the

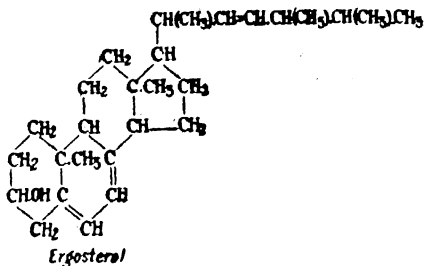
name (Gk. *chole*-bile). A derivative of cholesterol, 7-dehydro-cholesterol, is normally present in the skin. On exposure to sunlight it is converted into vitamin D_2 . Cholesterol is not, however, found in the vegetable kingdom. It crystallizes from hot aqueous alcohol with one molecule of water of crystallization in four sided plates with characteristic notched angles and from ether or benzene in needles; m.p. 145° ; $[\alpha]_D = -31.6^\circ$ (chloroform); $[\alpha]_D = -31.1^\circ$ (ether). It is easily soluble in ether, acetone, chloroform, and benzene. It is insoluble in water, slightly soluble in cold alcohol but easily soluble in hot alcohol. It is soluble in bile salts but insoluble in aqueous acids or alkalis. The melting points of the acetyl derivative (114°) and the benzoyl derivative (145°) help to differentiate cholesterol from the phytosterols. It forms a weak molecular union with digitoxin, saponin, etc. *Tests*: (1) If a solution of cholesterol in chloroform is shaken with the same volume of conc. H_2SO_4 , the chloroform solution is coloured red while the sulphuric acid shows a green fluorescence (Salkowski's reaction); (2) If one adds about 10 drops of acetic anhydride and one drop of conc. H_2SO_4 to about 10 drops of a solution of cholesterol in dry chloroform, the liquid changes in colour from rose to violet, then blue to dark-green (Liebermann-Burchard reaction).

Sitosterol, $C_{27}H_{48}.OH$. This is the *phytosterol* found in the grains of wheat and rye and in other vegetable seeds. It is a secondary alcohol with one double bond. From aqueous alcohol, it crystallizes with one molecule of water of crystallization in colourless plates, and from ether in needles; m.p. 137.5° ; $[\alpha]_D = -26.71^\circ$ (ether). It is easily soluble in ether, chloroform and benzene, insoluble in water, slightly soluble in cold alcohol, but easily soluble in hot alcohol. The acetyl derivative melts at 127.5° and the benzoyl derivative melts at 144.5° . The Salkowski and Liebermann-Burchard reactions are similar to cholesterol.

Ergosterol, $C_{28}H_{44}.OH$.

This occurs in very small amounts along with cholesterol in animal tissues and was first isolated from *ergot* (whence the name),

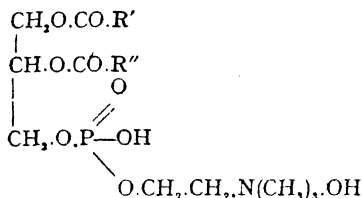
the fungus *Claviceps purpurea* which grows in rye. The main source of ergosterol is yeast from which it is usually prepared. It is an unsaturated (with double bonds) secondary alcohol with the complex structure shown here. It is changed into the antirachitic vitamin D₂ (calciferol) when it is exposed to *ultra-violet* rays, and ergosterol isolated from yeast is converted into vitamin D₂ (see p. 388) in the laboratory by exposing its ethereal solution to *ultra-violet* rays of definite wave-lengths. It is a colourless crystalline substance, m.p. 165°. It is soluble in ether and other fat solvents but insoluble in water.



Phosphatides, Phospholipins, Phospholipoids

These are compounds of phosphoric acid with fatty acids and nitrogenous substances, the best examples being the lecithins, the sphingomyelins, etc.

Lecithins. These are esters of glycerol with two fatty acids and phosphoric acid combined with the nitrogenous base choline $\text{HO}\cdot\text{CH}_2\text{CH}_2\cdot\text{N}(\text{CH}_3)_3\cdot\text{OH}$. The general formula for lecithins is as follows:



where $\text{R}'\text{CO}$. and $\text{R}''\text{CO}$ are two higher fatty acid radicals, at least one of which is unsaturated. The lecithins are found in the yolk of egg, in the liver, blood and other tissues, and they are also found in small amounts in vegetable tissues. They are yellowish-white amorphous substances, soluble in absolute alcohol, ether, petroleum ether, chloroform and benzene but almost insoluble in acetone.

Sphingomyelins. These are compounds of phosphoric acid, two fatty acids and the base choline and an unsaturated compound called sphingosin. They are found in fair amounts in brain tissues. It is not a true fat as it contains no glycerol.

Galactolipoids, Glycolipoids, Cerebrosides. These are compounds of galactose, a fatty acid and a nitrogenous base. They are found in the brain and nerve, tissues, e.g., phrenosin, kersin, nervon, etc., which contain sphingosin as one of their constituents.

CHAPTER XV

DI- AND POLYBASIC ACIDS, ALDEHYDIC, KETONIC AND HYDROXY ACIDS. OPTICAL ISOMERISM

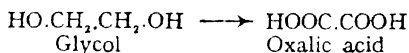
Saturated Dibasic Acids.

The acids of this series contain two carboxyl groups. The lowest member is known as oxalic acid and hence these acids are known as *acids of the oxalic series*. They form the following *homologous series*, the *general formula* being $\text{HOOC.C}_n\text{H}_{2n}\text{.COOH}$:—

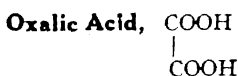
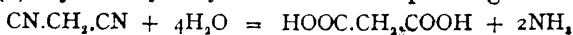
Oxalic acid HOOC.COOH
 Malonic acid $\text{HOOC.CH}_2\text{.COOH}$
 Succinic acid $\text{HOOC.}(\text{CH}_2)_2\text{.COOH}$
 Glutaric acid $\text{HOOC.}(\text{CH}_2)_3\text{.COOH}$
 Adipic acid $\text{HOOC.}(\text{CH}_2)_4\text{.COOH}$
 Pimelic acid $\text{HOOC.}(\text{CH}_2)_5\text{.COOH}$
 Suberic acid $\text{HOOC.}(\text{CH}_2)_6\text{.COOH}$
 Azelaic acid $\text{HOOC.}(\text{CH}_2)_7\text{.COOH}$
 etc., etc.

General Methods of Preparation.

(1) By the oxidation of the corresponding dihydric alcohols: *e.g.*,



(2) By the hydrolysis of the corresponding nitriles: *e.g.*,

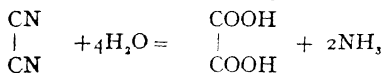


Occurrence.—The acid and its salts are known to occur in many plants; *e.g.*, in rhubarb roots (*Rheum emodi* Wall.) and in onion as insoluble Ca-oxalate, in rhubarb leaves and leaf-stalks and in the leaves of the Indian wood-sorrel or *amrul* (*Oxalis corniculata* Linn.) as acid potassium oxalate,

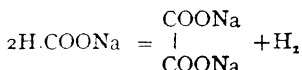
whence the name 'salt of sorrel' for the latter which is fairly soluble in water. Its salts occur in small amounts in normal urine but is increased (oxaluria) under certain metabolic disturbances. Oxaluria is frequently followed by or occurs along with glycosuria (diabetes). Its excretion in the urine is also increased by the use of vegetables containing excess of salts of oxalic acid such as, *Ole*, *Kachu* or *Arui* (*Arum*), onion, etc.

Synthesis

(1) By the hydrolysis of cyanogen or oxalo-nitrile:



(2) By heating sodium formate under reduced pressure to about 280° :



The free acid is liberated by dilute sulphuric acid and purified by crystallization from water. Since Na-formate (see formic acid) is obtained cheap by the action of carbon monoxide upon caustic soda, this method is now used for the preparation of oxalic acid on a large scale.

Preparation of Oxalic Acid.

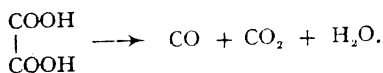
(3) *In the laboratory*, oxalic acid is easily prepared by oxidizing cane sugar with concentrated nitric acid. The cane sugar (15 g) is carefully heated in a flask over a water bath with conc. HNO_3 (75 c.c.) and when the reaction has ceased the solution is transferred to a basin and concentrated on the water bath to a small bulk when crystals of oxalic acid separate out on cooling. They are purified further by recrystallization from water.

(4) *On a large scale*, it is obtained by heating cellulose with caustic alkalies. Sawdust which is rich in cellulose is made into a paste with a strong solution of crude *caustic potash and caustic soda* and heated in thin layers over iron plates at 240° to 250° . The alkali oxalates formed are

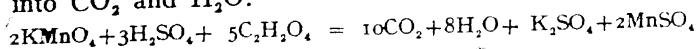
extracted with boiling water, filtered and concentrated. The oxalates which crystallize out are redissolved in boiling water and treated with milk of lime when calcium oxalate is precipitated. It is washed with water and decomposed with the calculated amount of dilute sulphuric acid, the insoluble CaSO_4 being removed and crystals of oxalic acid obtained on concentration. It is further purified by recrystallization from water.

Properties, Reactions and Uses of Oxalic Acid.

Oxalic acid crystallizes from water in colourless prisms with 2 molecules of water of crystallization $\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$. The anhydrous acid can be obtained by heating the hydrate at 100° or by keeping it over conc. H_2SO_4 . The hydrated acid melts at 101.5° whilst the anhydrous acid melts at 189.5° . Oxalic acid is fairly soluble in water and alcohol but only slightly in ether, and it is insoluble in petroleum ether, chloroform and benzene. When heated gradually, the water of crystallization is first given off; the acid then melts and a portion sublimes; on further heating it is decomposed into formic acid and CO_2 ; the formic acid also decomposes partially into CO and H_2O . When heated with conc. H_2SO_4 there is no charring but it is decomposed into CO and CO_2 :



When warmed with potassium permanganate in presence of dilute sulphuric acid, oxalic acid is oxidized quantitatively into CO_2 and H_2O :



Pure oxalic acid is, therefore, used for standardization of solutions of potassium permanganate. Oxalic acid is also used in making metal polishes and inks. As a dibasic acid it gives both normal and acid salts. The normal Na-, K- and NH_4 -salts are soluble but most of the other normal salts are sparingly soluble in water. Acid potassium oxalate KHC_2O_4 is sparingly soluble in water; it is used as a mordant in dyeing. Calcium oxalate is insoluble in water and in acetic acid but soluble in dilute mineral acids; this serves to distinguish oxalic acid from many other acids. The double

salt, potassium quadroxalate or tetroxalate $\text{KHC}_2\text{O}_4 \cdot \text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$, also known as "salts of lemon" or "salts of sorrel" is used in removing ink stains and iron moulds, the tannate of iron being soluble in the oxalate solution. Potassium ferrous oxalate $\text{FeK}_2(\text{C}_2\text{O}_4)_2 \cdot \text{H}_2\text{O}$ is used as a developer in photography owing to its strong reducing properties.

Both oxalic acid and its acid salts are powerful corrosive poisons (see Toxicology, pp. 444-446).

Other Saturated Dibasic Acids.

Malonic Acid, $\text{CH}_2 \begin{matrix} \swarrow \text{COOH} \\ \searrow \text{COOH} \end{matrix}$: Occurs in sugar beet as calcium

salt; colourless crystalline solid, m.p. 132° ; soluble in water, alcohol and ether; the ethyl ester is widely used in the synthesis of monobasic and dibasic acids and other organic compounds such as barbituric acid.

Succinic Acid, $\begin{matrix} \text{CH}_2 \cdot \text{COOH} \\ | \\ \text{CH}_2 \cdot \text{COOH} \end{matrix}$: Occurs in unripe grape, goose-

berry, currant, apple, banana, rhubarb, etc., and in various plants; obtained by the dry distillation of *amber*, a fossil resin; formed during the alcoholic fermentation of sugars; colourless prisms, m.p. 185° ; easily soluble in water; the calcium salt is soluble in water.

Glutaric Acid, $\text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$: occurs in unripe sugar beet, in sheep's wool, etc.; colourless crystals, m.p. 97.5° ; soluble in water, alcohol and ether.

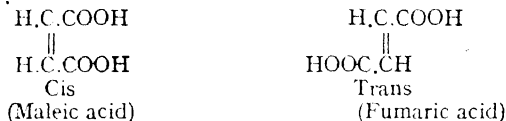
Unsaturated Dibasic Acids.

Fumaric Acid, $\text{HOOC} \cdot \text{CH} : \text{CH} \cdot \text{COOH}$; occurs in the plant *Fumaria officinalis* L., whence the name, in various fungi, etc.; prepared by heating malic acid to $140\text{--}150^\circ$; m.p. 286.7° ; when heated to about 200° , fumaric acid sublimes and is converted into the anhydride of its stereoisomer maleic acid.

Maleic Acid, $\text{HOOC} \cdot \text{CH} : \text{CH} \cdot \text{COOH}$: this does not occur in nature; m.p. 130° ; prepared by boiling maleic anhydride, obtained from fumaric acid, with water.

The kind of isomerism typified by these two acids is known as *geometrical isomerism*. The structure assigned to them show a difference in the distribution of the atoms or groups of atoms in space. In the *cis* form the COOH groups are close together and in

the *trans* form they are further apart. The structural difference is shown below:

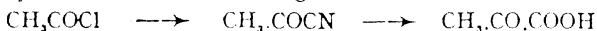


Other examples of geometrical isomerism have been mentioned already amongst unsaturated monobasic acids, e.g., oleic acid and elaidic acid, etc. (see p. 153).

Aldehydic Acids, e.g., Glyoxylic Acid, Glyoxalic Acid, CHO.COOH , prepared by reducing oxalic acid, with magnesium amalgam; it is a syrupy liquid, soluble in water; it gives reactions for both acid and aldehyde.

Ketonic Acids.

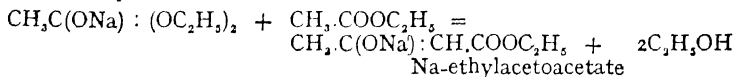
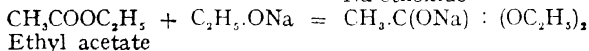
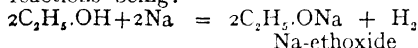
Pyruvic Acid, *Keto Propionic Acid*, $\text{CH}_3\text{CO.COOH}$: an intermediate product in the metabolism of fatty acids and amino acids, as well as in the transformation of glucose into alcohol or lactic acid; may be synthesised in the following manner:



prepared by distilling tartaric acid with potassium bisulphate; liquid, b.p. 168° ; soluble in water; smells like acetic acid; shows the properties of both acids and ketones.

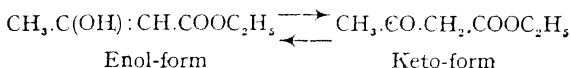
Acetoacetic Acid, *Diacetic Acid*, β -Ketobutyric Acid, $\text{CH}_3\text{CO.CH}_2\text{COOH}$: It is the product of the oxidation of β -hydroxybutyric acid and appears in the blood and urine of diabetic patients. It can be best prepared by carefully hydrolyzing ethyl acetoacetate with dilute KOH in the cold, acidifying with dilute sulphuric acid and extracting the free acid with ether. It is a thick liquid with a strong acid reaction miscible with water; decomposes easily by heat (even at 100°) into acetone and carbon dioxide. It gives a reddish violet colour with ferric chloride (*Gerhardt's test*). Rothera's N-nitro-prusside test used for acetone has been shown to be more delicate for this acid.

Ethyl Acetoacetate, $\text{CH}_3\text{CO.CH}_2\text{COOC}_2\text{H}_5$: obtained by the action of metallic sodium on dry ethyl acetate containing some dry alcohol, the reactions being:



The sodium derivative is decomposed with dilute acetic acid, poured into a saturated solution of NaCl and the ester is separated; it is purified by distillation.

It is a colourless liquid with a pleasant smell; b.p. 180° ; sp. gr. 1.0246 at 20° ; slightly soluble in water, more easily soluble in the usual organic solvents; used in the synthesis of antipyrine and various other organic compounds. It exists in the following *tautomeric forms*, the ordinary ester being an equilibrium mixture containing about 7.5 per cent of the enol form:



Hydroxy Acids

Lactic acid $\text{CH}_3\text{CH}(\text{OH}).\text{COOH}$ is called α -hydroxy propionic acid because the OH group is attached to the carbon atom next to the COOH group, the formula for β -hydroxy propionic acid would, therefore, be $\text{CH}_2(\text{OH}).\text{CH}_2.\text{COOH}$, and the formula for γ -hydroxy butyric acid would be $\text{CH}_2(\text{OH}).\text{CH}_2.\text{CH}_2.\text{COOH}$, and so on. Polybasic hydroxy acids like tartaric acid and citric acid are also discussed under this head.

Lactic Acid. α -Hydroxy propionic Acid,
 $\text{CH}_3\text{CH}(\text{OH}).\text{COOH}$

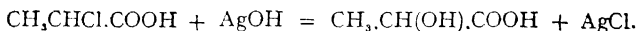
Occurrence—Lactic acid occurs in three forms. The optically inactive form (racemic or dl-lactic acid, also known as fermentation lactic acid) occurs in sour milk as the fermentation product of milk sugar or lactose, whence the name lactic; also occurs in the gastric juice in certain types of dyspepsia and in malignant disease (cancer) of the stomach but occurs normally in the gastric juice of cattle.

The optically active isomer (d-lactic acid) known as para- or sarcolactic acid (*Gk.* *sarcos*—flesh) occurs in muscles and is increased by muscular activity.

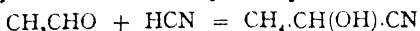
The other optically active isomer (l-lactic acid) does not occur in nature.

Synthesis and Preparation of Lactic Acids.

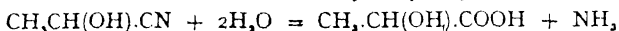
(1) By the action of moist silver oxide on chloropropionic acid:



(2) From acetaldehyde, by the following reactions:



Aldehyde cyanhydrin or Lacto-nitrile



(3) *On a large scale*, the dl-lactic acid is prepared by the *lactic acid fermentation* of sugars, such as cane sugar, glucose, etc., with the help of special strains of lactic acid bacillus. Molasses may also be used for this purpose.

(4) This *biological method* can be used in the laboratory in the following manner: Cane sugar (25g) is dissolved in water (250 c.c.) and sour milk (20 c.c.), which contains the lactic acid bacillus, is added. The solution is kept between 45°-55° to prevent alcoholic and butyric fermentations, and powdered chalk (10g) is stirred in from time to time to neutralize the acid formed, since even 1 per cent of the free acid inhibits the action of the bacillus. When all the sugar disappears (8 to 10 days) the solution is boiled to kill the bacteria, filtered and concentrated on the water bath. The calcium lactate which crystallizes out is separated and recrystallized from hot water. It is dried, the free acid liberated by adding the calculated amount of dilute sulphuric acid, the calcium sulphate filtered off and the lactic acid obtained either by extraction with ether or by distillation under reduced pressure. Instead of CaCO_3 , zinc carbonate may also be used and in that case the zinc is removed by H_2S .

(5) The d-lactic or sarcolactic acid is usually prepared from commercial meat extract (Liebig's meat extract); it may also be obtained by the action of *Penicillium glaucum* on dl-lactic acid in which it grows at the expense of its lævo-component.

(6) The l-lactic acid is obtained by the action of *Bacillus acidilævo-lactici* on a solution of cane sugar and also by the resolution of the dl-lactic acid with the base strychnine, the first salt to crystallize out on fractional crystallization, being the strychnine-l-lactate.

Properties and Reactions of Lactic Acid.

Lactic acid is a colourless liquid with a sour taste and smell. dl-Lactic acid melts at 18° and d- or l-lactic acid melts

at 26°. Lactic acid may be distilled without decomposition in high vacuum; b.p. 82°-85° (0.5 to 1 mm.), 119° (12 mm.). It has a sp. gr. of 1.248 at 15°. It is hygroscopic and easily soluble in water and alcohol, less so in ether. It is slightly volatile with steam but volatilizes more easily with superheated steam; partially decomposed on heating into acetaldehyde and carbon monoxide:



With strong sulphuric acid this reaction takes place more readily.

The specific rotation of d-lactic acid, $[\alpha]_D^{15} = +3.82^\circ$ (conc. = 10.458 in water) but the rotation changes with the concentration. *Calcium lactate* $\text{Ca}(\text{C}_3\text{H}_5\text{O}_3)_2 \cdot 4\frac{1}{2}\text{H}_2\text{O}$ crystallizes from water; soluble in 12.4 parts of cold water, easily soluble in boiling water or alcohol; the salt prepared from the dextro acid is laevorotatory $[\alpha]_D = -3.87^\circ$ (c = 7.23 in water). *Zinc lactate* $\text{Zn}(\text{C}_3\text{H}_5\text{O}_3)_2 \cdot 2\text{H}_2\text{O}$ crystallizes from water; one part dissolves in 17.5 parts of water at 15°; the salt prepared from the dextro acid is laevorotatory $[\alpha]_D^{15} = -6.00^\circ$ (c = 5.0 in water); -8.0° (c = 2.5 in water) for the anhydrous salt; the salt prepared from the laevo acid is dextrorotatory. These properties of the Ca- and Zn-salts are utilized in separating and identifying lactic acid formed during biochemical changes.

Uses.—Lactic acid is used for removing lime from hides in the tanning industry. Some of its salts are used in dyeing and in calicoprinting. Calcium lactate is used in medicine in calcium deficiency. Ethyl lactate is used as a solvent in the lacquer industry.

Tests for Lactic Acid.

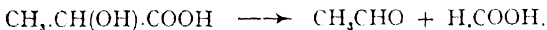
(1) *Hopkins Thiophene Test.* 5 c.c. of conc. H_2SO_4 , three drops of a saturated solution of copper sulphate and a few drops of the lactic acid solution are shaken together, heated over a boiling water bath for 5 minutes, cooled, and 2-3 drops of a half per cent solution of thiophene in alcohol are added; on warming, a cherry red colour is developed.

(2) If a very dilute solution (almost colourless) of ferric chloride is added to a solution of lactic acid as also to other hydroxy acids it is coloured yellow owing to the formation of a deep yellow ferric salt.

(3) A two per cent solution of phenol is treated with a dilute solution of ferric chloride until there is a distinct violet colour; on adding a dilute solution of lactic acid, the colour becomes yellow (Uffelmann's test).

(4) *Lactic acid gives the iodoform reaction.*

(5) *Micro Tests*. A few milligrams of the acid are distilled with a mixture of one volume of conc. H_2SO_4 and two volumes of water, and half the distillate is tested for acetaldehyde by the phenylhydrazine method and the other half for formic acid by cerium nitrate:



The crystalline structure of cobalt lactate, obtained by adding cobalt nitrate to an alkali salt, as tufts of reddish needles or of zinc lactate (colourless little rods, platelets, stars or x-forms) are characteristic.

β -Hydroxy Butyric Acid, $\text{CH}_3\text{CH}(\text{OH})\text{CH}_2\text{COOH}$: The *lævo* acid occurs in the blood of diabetic patients and is stated to be excreted in the urine in fair amounts in severe cases of diabetes; colourless, hygroscopic, crystalline substance, easily soluble in water, ether and alcohol, insoluble in benzene; m.p. $49-50^\circ$; $[\alpha]_{\text{D}}^{16} = -24.9^\circ$ (conc. = 8.33 in water).

Acetone Bodies.

The acetone bodies or *ketone bodies* which are excreted in urine of diabetic patients consist of acetone, aceto acetic acid and β -hydroxybutyric acid. They indicate incomplete combustion of fats and accumulation of acids in the system giving rise to what is called *acidosis* or *ketosis*. Normally β -hydroxy butyric acid, which is formed by the oxidation of higher fatty acids, is oxidized to aceto acetic acid:



The latter again undergoes ketonic hydrolysis and is decomposed into acetone and CO_2 :



The acetone is finally oxidized to CO_2 and H_2O . But in abnormal conditions, they are not completely burnt up and appear in the urine.

Stereoisomerism and Optical Activity

We have seen that two kinds of lactic acid occur in nature, the fermentation or racemic or dl-lactic acid and sarcolactic acid or d-lactic acid. It has also been mentioned that dl-lactic acid can be resolved into d-lactic acid and l-lactic acid by crystallizing the strychnine salt. The dextro-lactic acid and the *lævo*-lactic acid have exactly the same molecular weight, the same structural formula and the same chemical

properties. They agree in most of their physical properties but differ in one most important physical property, namely, in their *optical activity*. This sort of isomerism is designated as *optical isomerism*. They differ not only in optical activity but, for compounds which possess physiological action, show a difference in their *physiological action* as well (see below).

The difference in optical activity has been explained independently and almost simultaneously by Van't Hoff and Le Bel by the difference in arrangement of the atoms or radicals in space. The carbon atom is assumed to occupy the centre of a *regular tetrahedron*, a solid bounded by four equilateral triangles (Fig. 28), and the four valencies are directed towards the four corners of the tetrahedron. Or, in

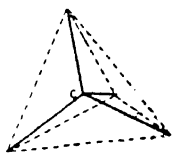


FIG. 28.

other words, the four atoms or groups of atoms attached to a carbon atom are distributed symmetrically in space and do not occupy the same plane. If the four valencies are satisfied with four different atoms or radicals (groups of atoms), *i.e.*, if the *carbon atom* is *asymmetric* as we see in lactic acid, we may expect two arrangements which cannot be superimposed one upon the other (Fig. 29), perhaps best illustrated by the *right hand* and the *left hand* or by an *object* and its *mirror image*. The one corresponds to the *dextro-form* (d-) and the other to the *laevo-form* (l-). A mixture of the two in equal quantities would be the *inactive*-(dl-) or racemic form.

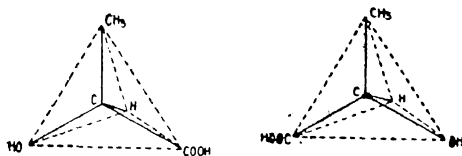


FIG. 29.

As has been mentioned above this sort of isomerism is known as *optical isomerism*. It is a special case of *stereoisomerism* (Gk. *stereos*—solid), and may be expected in every compound containing one or more *asymmetric carbon atoms*.

N.B. Optical isomerism is also known amongst compounds containing other polyvalent asymmetric atoms such as N, S, etc.

The number of optical isomers goes on increasing with the number of asymmetric C atoms. For instance, one asymmetric C atom gives 2^1 or 2 isomers, 2 asymmetric C atoms give 2^2 or 4 isomers, 3 asymmetric C atoms give 2^3 or 8 isomers (e.g., pentoses), 4 asymmetric C atoms give 2^4 or 16 isomers (e.g., hexoses), and so on.

Resolution of an Optically Inactive Compound into its Active Components.—

(1) By the *fractional crystallisation of the salt* prepared from the dl-acid or base with an optically active base or acid. For example, dl-tartaric acid forms salts with the optically active base quinine (lævo-rotatory) and when fractionally crystallized, quinine-d-tartrate crystallizes out first since it is less soluble than quinine-l-tartrate. The d-acid or l-acid can then be easily liberated from the salts. (2) By *mechanically sorting out* the two types of crystals in a salt. Thus, the sodium-ammonium salt obtained from dl-tartaric acid is found to consist of two types of crystals, the one corresponding to the dextro-acid and the other to the lævo acid, and they can be sorted out if the crystals are sufficiently large or with the help of a hand-lens. (3) By the *destruction of one of the isomers, with certain micro-organisms*. Thus, if the ammonium salt of dl-tartaric acid is acted upon by *Penicillium glaucum*, it is found that the salt of the dextro acid is destroyed leaving the lævo salt intact. In the case of dl-lactic acid, the lævo salt is destroyed leaving the d-salt intact.

Difference in Physiological Action between Dextro and Laevo Compounds.—

d-Asparagine is sweet to taste while l-asparagine is insipid; l-nicotine is much more toxic than d-nicotine; l-hyoscyamine has a more powerful mydriatic action than d-hyoscyamine; l-adrenaline has a much stronger pressor action than d-adrenaline; and so on.

POLARIMETER

The optical activity of a substance is measured by an instrument called the polarimeter. The essential component of the instrument is the *Nicol prism* which has the property of polarizing light and is made from a naturally occurring transparent crystal of calcium carbonate (Calc Spar or Iceland Spar) cut out in a particular manner.

As is well known, waves of light vibrate transversely to the direction of the ray of light and the *plane of vibration* of ordinary light takes all possible positions about the line of propagation. Now the Nicol prism has the property of fixing the plane of vibration of light; once the light passes through the prism the plane of vibration is fixed to one definite plane and the light is said to be *plane polarized* or simply *polarized*. The Nicol prism next to the source of light is therefore known as the *polariser*.

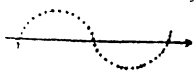


FIG. 30.

In a polarimeter, the rays of light from the source L, made parallel by lenses (Fig. 31), first pass through the Nicol prism P known as the *polarizer* and then through a quartz plate Q (or a smaller Nicol prism) which covers half the field of vision. The rays next pass through the tube T containing the solution to be tested, then through the second Nicol prism A known as the *analyzer* and finally through a combination of lenses which focus the eye on to the quartz plate (or the smaller Nicol). The polarizer is fixed while the analyzer can be rotated by means of a handle, and the angle through which it is turned can be read very accurately on a graduated scale engraved on a circular metal disc with the help of a vernier.

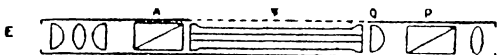


FIG. 31.

The polarized rays that emerge from the polarizing Nicol fall on the face of the analyzer and will only pass through unimpaired provided they can contrive to vibrate in the same plane. In this position the Nicols are said to be *parallel*. The case is similar to that of a thin knife blade passing through two thin slits (Fig. 32). If the analyzer be rotated from the above position through an angle of 90° , the rays are completely absorbed, the field will appear dark and the Nicols are said to be *crossed*. On rotation through a further angle of 90° the Nicols will be again parallel.



FIG. 32.

If the two Nicol prisms are so arranged that light may pass through, i.e., if the Nicols are parallel, and if a solution of an optically active substance is interposed between them, the light does not pass any more as before. The solution has turned the plane of polarization, and the analyzer must now be turned either towards the right or towards the left to allow the same amount of light to pass through. If the solution has turned the plane towards the right (i.e. if the analyzer has to be rotated to the right) the substance is said to be *dextro-rotatory* and if to the left, *laevorotatory*.

If there is nothing to intervene between the polarizer and the analyzer, as we rotate the analyzer through 180° in either direction, the light decreases from a maximum to a minimum and then increases

to a maximum again. It will thus be very difficult to judge the position when the field is almost dark and still more so for the maximum light. To avoid this difficulty, a thin *quartz plate* (as in the Laurent type of instrument) or a small *Nicol prism* (as in the Lippich type of instrument), covering half the field of vision, is interposed between the polarizer and the solution. Now quartz (or Nicol prism) is an optically active solid which rotates the plane of polarization of polarized light. If the analyzer is crossed with regard to the polarizer, the whole field should appear dark but when the quartz plate (or Nicol prism) is interposed, half the field covered by this becomes illuminated. If the analyzer is turned so that this half of the field appear dark then the other half becomes bright. A position intermediate between these two points can be found out by trial at which both the halves of the field appear equally illuminated. This position of equal but feeble illumination is taken as the *zero-reading* of the instrument. The difference between the second position of feeble but equal illumination (when the optically active substance is interposed) and the zero reading gives the observed angle of rotation (Fig. 33).



FIG. 33.

N.B. The small Nicol prism used in the Lippich instrument has this advantage over the *quartz plate* in the Laurent type that the former can be used with monochromatic light of any wave length whereas the quartz plate can be used only for monochromatic sodium light.

The *source of light* is generally a *gas burner* over which a loop of platinum is placed and this is fed with fused NaCl in order to get the yellow D line of Na (wave length 5890 Å). In modern instruments, an *electric bulb containing metallic sodium* is supplied, and in costly instruments one may also find an ordinary electric bulb with a *spectroscopic arrangement* for use with any wave length of light.

The solution or the pure liquid is taken in a narrow glass *tube* closed with a glass disc on either side and it is so arranged that the length of the solution exactly measures either one decimetre or a fraction or a multiple of the same.

The *observed angle of rotation* through which the plane of polarization is turned depends upon several factors, viz., (a) the nature of the substance dissolved, (b) the nature of the solvent, (c) the length of the column of the solution, (d) the concentration of the solution, (e) the temperature of the solution, (f) the wave length of the source of light used, etc. But although the observed angle of rotation varies with these factors, the *specific rotation* of a substance has a definite value under certain standard conditions and it has, therefore, proved to be very useful.

The *specific rotation* of a substance is defined as the rotation of polarized light produced by a solution containing one gram of the substance in one cubic centimetre of the solution in a layer one decimetre in length. In the case of an optically active liquid, the specific rotation of the *pure liquid* is defined as the rotation produced by a column of the liquid one decimetre long divided by the density of the liquid.

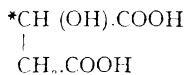
The specific rotation is represented by the symbol $[\alpha]_D^t$ in which α represents the observed angle of rotation, D represents the D line of sodium light and t the temperature, and this is related to the observed angle of rotation by the equation:

$$[\alpha]_D^t = \frac{\alpha \times 100}{c \times l}$$

where α is the observed angle of rotation, c is the concentration of the solution, i.e., grams of substance contained in 100 c.c. of the solution, and l is the length of the column of the solution in decimetres.

Applications of the Polarimeter. The instrument helps us (1) to find out whether a substance is optically active or not, i.e., if it contains an asymmetric carbon atom, (2) to identify a substance by its specific rotation, (3) to detect the amount of adulteration of an optically active substance, (4) to study biochemical reactions, such as enzyme action, etc.

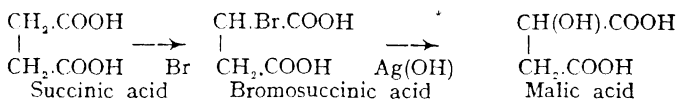
Malic Acid, Monohydroxy Succinic Acid,



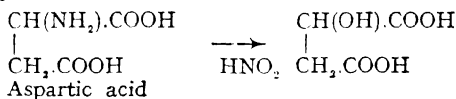
Occurrence. It occurs in nature in many unripe fruits such as apples (*L. malum*—apple, whence the name), grapes, currants, cherries, etc., either in the free state or as the potassium or calcium salt.

Synthesis.

(1) By treating bromosuccinic acid with moist silver oxide:



(2) By the action of nitrous acid on aspartic acid:



(3) *Preparation.* The juice obtained by pressure or the extract obtained by boiling the unripe apples with water, is treated with milk of lime. The neutral calcium salt of malic acid which is precipitated is separated by filtration and washed with cold water. It is dissolved in a small amount of hot dilute (1 in 10) nitric acid and filtered hot. On cooling, crystals of acid calcium malate $\text{Ca}(\text{C}_4\text{H}_5\text{O}_5)_2 \cdot 6\text{H}_2\text{O}$ separate out. This is further purified by recrystallization from water. The pure acid calcium malate is decomposed by the calculated amount of dilute sulphuric acid and filtered. On concentration crystals of malic acid separate out.

Properties and Reactions. Malic acid forms colourless hygroscopic crystals soluble in water and alcohol. From the above formula it is clear that it contains an asymmetric carbon atom (marked with an asterisk) and so it may occur in two optically active forms. The acid that occurs in nature is the lævo variety (l-), whereas the synthetic acid is inactive (dl-). Lævo malic acid melts at 100° whereas the dl-or racemic acid melts at 130° .

On heating, malic acid loses water and is converted into a mixture of maleic and fumaric acids (see p. 177):

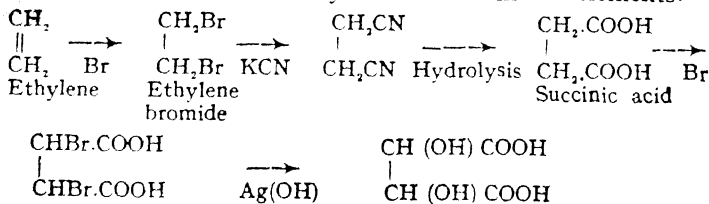
Tartaric Acid, Dihydroxy Succinic Acid, $\text{CH}(\text{OH})\cdot\text{COOH}$

$\text{CH}(\text{OH})\cdot\text{COOH}$

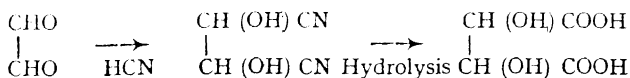
Occurrence. Tartaric acid occurs in many fruits such as tamarinds, grapes, tomatoes, pineapples, etc., partly as the free acid and partly as K or Ca salt. Grapes and tamarinds are particularly rich in this acid and the former constitutes an important natural source for its preparation on a large scale.

Synthesis.

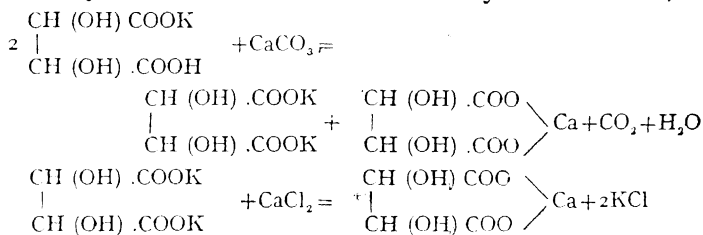
(1) By the action of moist silver oxide on dibromosuccinic acid which can be synthesized from the elements:



(2) By the action of hydrocyanic acid on glyoxal and subsequent hydrolysis of the cyanogen derivative or the nitrile of tartaric acid:



(3) *Preparation of Tartaric Acid from Grape Juice.* During the fermentation of grape juice, the crude acid potassium tartrate (which is only slightly soluble in water and much less soluble in the dilute alcohol produced by the fermentation) separates out in small reddish-brown crystals. This is known as *argol*. The pure acid potassium tartrate $\text{HOOC} \cdot \text{CH (OH)} \cdot \text{CH (OH)} \cdot \text{COOK}$ obtained from argol by recrystallization from hot water is known as *cream of tartar*. To prepare tartaric acid, the argol is dissolved in hot water and neutralized with chalk. The precipitated calcium tartrate is filtered off and the filtrate containing neutral potassium tartrate is treated with calcium chloride. The total calcium tartrate thus obtained is separated, decomposed with the calculated quantity of dilute sulphuric acid, filtered from calcium sulphate, the filtrate decolorized by animal charcoal, and crystals of tartaric acid obtained by concentration.



The acid obtained from grape juice and other fruit juices is the dextrotartaric acid.

Properties

Dextro-tartaric acid crystallizes in large colourless transparent prisms. When heated rapidly it melts at about 170° . It dissolves easily in water and alcohol but is insoluble in ether. Dextro-tartaric acid shows the specific rotation: $[\alpha]_D^{20} = +14.00 - 0.1316^\circ \text{c}$, where c , the concentration, ranges from 2.5 per cent to 25 per cent; the rotation

changes both with the temperature and the concentration of the solution. When heated with water in a sealed tube to 175° , d-tartaric acid is partially converted into *racemic* acid, and when heated to 165° in the same way it is partially converted into *mesotartaric* acid (see later). When dry tartaric acid is heated in a test tube it chars and emits an odour of burnt sugar. Tartaric acid is a strong reducing agent and reduces ammoniacal silver nitrate and Fehling's solutions.

Uses. The free acid or cream of tartar is used as a ingredient of effervescent powders such as *Siedlitz powder* (a purgative), baking powder, etc. Sodium potassium tartrate, $\text{NaOOC}(\text{OH})\text{CH}.\text{CH}(\text{OH}).\text{COOK} \cdot 4\text{H}_2\text{O}$, known as *Rochelle salt* or *Seignette salt* from the name of the discoverer Seignette de la Rochelle, is prepared by neutralizing cream of tartar with sodium carbonate. Rochelle salt is used in preparing *Fehling's solution* (see p. 203), since it has the property of preventing the precipitation of cupric hydroxide from an alkaline medium probably due to the for-

mation of the complex copper derivative

$$\begin{array}{c} \text{NaOOC} \cdot \text{CH} \cdot \text{O} \\ | \\ \text{KOOC} \cdot \text{CH} \cdot \text{O} \end{array} \bigg\rangle \text{Cu}.$$

Rochelle salt is also used in silvering glass for mirrors. Potassium antimonyl tartrate or *tartar emetic* $\text{KOOC}(\text{OH})\text{CH}.\text{CH}(\text{OH}).\text{COO}(\text{SbO}) \cdot \frac{1}{2}\text{H}_2\text{O}$ is prepared by boiling a solution of acid potassium tartrate with antimonious oxide. It is a colourless crystalline substance soluble in water and used in medicine as an emetic and also as a mordant in dyeing and calico printing. Both tartar emetic and the corresponding sodium salt are used in the treatment of Kala-azar and bilharziasis.

Tests.

(1) On adding silver nitrate to a neutralized solution of tartaric acid, there is a white precipitate of silver tartrate which redissolves in ammonia and produces on warming in a water bath a bright silver mirror on the sides of the test tube.

(2) On adding calcium chloride to a concentrated neutral solution of a tartrate a white precipitate of calcium tartrate is produced in the cold; the precipitate is soluble in acetic acid.

(3) *Fenton's Test*; To 2 c.c. of a neutral solution of a tartrate add a drop of a freshly prepared dilute solution of ferrous sulphate:

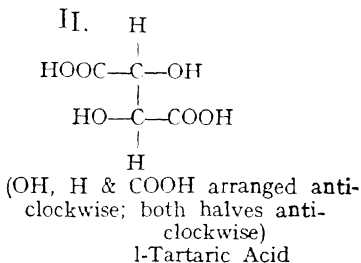
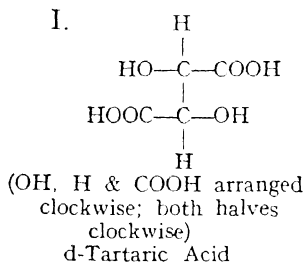
add 2 drops of a dilute solution of hydrogen peroxide and then some dilute NaOH; there is an intense violet colour due to the formation of the ferric salt of dihydroxymaleic acid.

(4) *Denige's Test*: Add 2 drops of a solution of a tartrate to 2 c.c. of conc. H_2SO_4 containing 2 drops of a 2 per cent solution of resorcinol and warm; a violet colour develops.

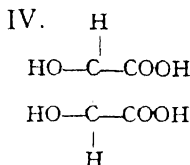
Stereoisomerism of Tartaric Acid.

The formula of tartaric acid shows that there are two similar asymmetric carbon atoms in the molecule. It exists in two optically active forms, the *dextro* and *laevo* acids, one being the mirror image of the other.

A mixture of d- and l-acids in equal quantities would be the inactive (racemic or dl-) acid. There is also another possibility. Since the atoms or groups of atoms attached to the two asymmetric carbon atoms are similar, the arrangement round one carbon atom may represent the *dextro* form and that round the other carbon atom the *laevo* form, one being the mirror-image of the other. The total effect will be an inactive acid which cannot be resolved into *dextro* and *laevo*-forms. This inactive acid is in fact found to occur and is called *meso-tartaric* acid. It is said to be inactive by *internal compensation* as distinct from the *racemic tartaric acid* which is inactive by external compensation and which can be resolved into the *dextro* and *laevo* forms. The four tartaric acids may thus be represented graphically as follows.

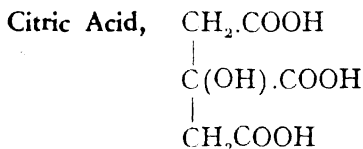


III. The mixture of I and II in equimolecular quantities is the racemic or dl- tartaric acid.



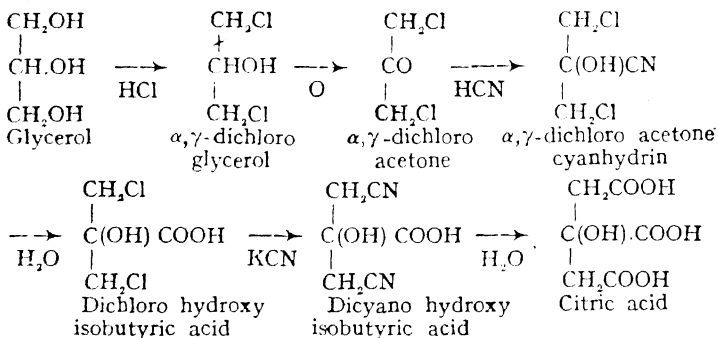
Meso-tartaric acid

(OH, H & COOH in upper half clockwise and in lower half anti-clockwise)



Occurrence. Citric acid occurs in the free state in fair amounts in fruits of the genus *Citrus*, *e.g.*, lemon, lime, orange, pampelo, etc. In lemon juice it may occur up to about 8 per cent and hence this serves as an important source for its preparation. It is also known to occur along with other organic acids in tomato, currant, *tepari*, gooseberry, etc.

Synthesis



Preparation of Citric Acid.

(I) *From Lemons.*—The lemon juice obtained from the unripe fruits is first boiled in order to coagulate the proteins. The liquid is concentrated, filtered, treated with milk of lime and boiled. The calcium citrate, which is less soluble in

boiling water than in cold water, is precipitated. It is filtered hot and washed with boiling water. The calcium salt is then decomposed with the calculated quantity of dilute sulphuric acid, filtered from calcium sulphate, decolorized with animal charcoal and concentrated, when crystals of citric acid separate out.

(2) *Biological Method.* About 10 per cent solution of glucose is fermented in acid medium by moulds such as *Aspergillus niger*, and the citric acid produced is separated as before by the calcium salt.

Properties. Citric acid crystallizes from water with one molecule of water of crystallization, $C_6H_8O_7 \cdot H_2O$, in large colourless prisms. It dissolves easily in water and alcohol but is almost insoluble in ether and chloroform. It has an agreeable acid taste. The hydrated acid melts at about 100° when rapidly heated; on heating slowly, it becomes anhydrous, at about 130° and the anhydrous acid melts smoothly at 153° . When heated more strongly, it chars but does not emit the smell of burnt sugar like tartaric acid. The calcium salt is less soluble in hot water than in cold and it is, therefore, thrown down on boiling; this distinguishes citric acid from many other vegetable acids. As there is no asymmetric carbon atom, the acid is optically inactive. It is a tribasic acid and, therefore, forms three kinds of salts.

Uses. Citric acid is used in confectionery and in synthetic fruit drinks. It is used as a mordant in dyeing and in calico printing. *Ferric ammonium citrate* as well as the sodium and potassium salts are used in medicine, and magnesium citrate is used as a laxative.

Tests.

(1) A neutral solution of citric acid produces a curdy white precipitate of silver citrate which is soluble in ammonia; on warming this solution in the water bath there is no reduction of the silver salt (distinction from tartrates); on prolonged boiling, however, some reduction takes place.

(2) *Denige's Test:* To 1 c.c. of a solution of mercuric sulphate (*Denige's reagent*), add 5 c.c. of a solution of citric acid (5 per cent) and 2 drops of a 1 per cent solution of $KMnO_4$; the permanganate is decolorized and there is a white precipitate of a double salt of basic mercuric sulphate and mercuric acetone dicarboxylate.

CHAPTER XVI

CARBOHYDRATES

Importance and Occurrence.

The carbohydrates form one of the three important groups (fats, carbohydrates and proteins) of compounds required for our food. They contain only C, H and O, and the name *carbohydrate* came from the fact that besides carbon they were found to contain H and O in the proportion met with in water, *i.e.*, 2:1 as seen, for example, in the case of glucose $C_6(H_2O)_6$, Sucrose $C_{12}(H_2O)_{11}$, etc. Later discovery of compounds such as rhamnose $C_6H_{12}O_5$, etc., have shown that the above relationship is not necessarily true in every case although it holds good in the majority of compounds. The group is a large one and is of great economic importance, since they are used not only as food but also for the manufacture of paper, explosive, textile, alcohol, etc.

The carbohydrates are found to occur widely distributed in plants where they are built up from the CO_2 of the air and H_2O under the influence of sunlight and chlorophyll; from the simple member (glucose) thus formed the more complex ones are elaborated. Some of the complex members like starch serve as reserve food materials of plants while others like cellulose form the basis of the cell walls or part of the plant structure itself. Carbohydrates also occur in the animal kingdom but to a less extent, *e.g.*, lactose in milk, glucose in blood and other tissues, glycogen in liver, muscles, etc.

General Tests for Carbohydrates.

1. *Molisch's Reaction*.—About 5 c.c. of the solution are taken in a test tube and 2 or 3 drops of alpha-naphthol solution (5 per cent in alcohol) are added and mixed carefully. About 3 c.c. of conc. H_2SO_4 are allowed to flow down the side of the inclined test tube to form a layer beneath the mixture. A violet or reddish violet zone is formed at the junction due to the formation of a colouring matter by the interaction of α -naphthol with ω -hydroxymethylfurfural produced from the carbohydrate. This test is given by all carbohydrates.

2. *Thymol Test*.—This is a modification of Molisch's test. To about 0.5 c.c. of the solution or a small quantity of the solid, are added 3 drops of 3 per cent alcoholic solution of Thymol, 5 c.c. of conc. HCl and about 2g. of solid NaCl (helps to boil quietly). Boiled over a small flame for 1-3 minutes—a carmine colour develops. All carbohydrates including cellulose give this reaction.

Egg albumin and other proteins also respond to this test on account of the presence of a sugar residue in the protein molecule.

Classification. The carbohydrates may be classified under the following heads:

I. **Monosaccharides** or *Simple Sugars*.

1. Biose $C_2H_4O_2$, e.g., Glycollic aldehyde.
2. Trioses $C_3H_6O_3$, e.g., Glyceric aldehyde, Dihydroxy acetone.
3. Tetroses $C_4H_8O_4$, e.g., Erythrose.
4. Pentoses $C_5H_{10}O_5$, e.g., Arabinose, Xylose.
Methyl pentoses, $C_6H_{12}O_5$, e.g., Rhamnose.
5. Hexoses $C_6H_{12}O_6$, e.g., Glucose, Galactose, Mannose, Fructose, etc.
6. Heptoses, Octoses, etc.

II. **Oligosaccharides** or *Compound Sugars*.

1. Disaccharides $C_{12}H_{22}O_{11}$, e.g., Sucrose, Lactose, Maltose.
2. Trisaccharides, Tetrasaccharides, etc.

III. **Polysaccharides**.

1. Pentosans $(C_5H_8O_4)_n$, e.g., Araban, Xylan, etc.
2. Hexosans $(C_6H_{10}O_5)_n$, e.g., Starch, Dextrin, Glycogen, Cellulose, Inulin, Mannan, etc.

IV. **Complex Polysaccharides**.

e.g., Hemicelluloses, Gums, Mucilages, Pectins, etc.

Class I. Monosaccharides or Simple Sugars.

The monosaccharides are so called because they cannot be split up by hydrolysis into simpler compounds. If the molecule contains two carbon atoms it is called a *biose*, if 3 carbon atoms a *triose*, if four a *tetrose*, and so on. But Karrer suggests that the number of oxygen atoms and not the carbon atoms should be the determining factor in such classifications. Rhamnose $C_6H_{12}O_5$, for instance, would not be called a hexose although it contains 6 carbon atoms, but a methyl pentose since it contains only 5 oxygen atoms.

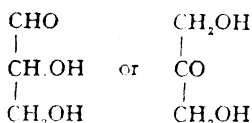
The lower members (up to the tetroses) do not occur in nature but they have been prepared synthetically. Some of the pentoses and many of the hexoses occur in nature whereas the heptoses, octoses and nonoses are only synthetic products. The monosaccharides, with the exception of the biose, glycollic aldehyde, can be regarded as *polyhydroxy-aldehydes* or *polyhydroxyketones* having 3 to 9 carbon atoms in the molecule. If the monosaccharide contains an aldehyde group, it is known as an *aldose*, and if a ketone group a *ketose*. They are sweet crystalline substances, soluble in water. As polyhydroxy compounds they form esters with acids such as phosphoric acid, acetic acid, nitric acid, etc., and form metallic derivatives similar to alcoholates (see glucose). As aldoses or ketoses, they show the characteristic reactions of aldehydes or ketones, such as the reduction of ammoniacal silver nitrate, Fehling's solution, etc., the formation of phenylhydrazones, osazones, cyanhydrins, oximes, etc. (see glucose).

I. Biose $C_2H_4O_2$



e.g., **Glycollic aldehyde** or Glycolose, CH_2OH : prepared synthetically; a colourless crystalline substance, m.p. $95-97^\circ$, with a sweet taste, easily soluble in water and hot alcohol but with difficulty in ether, reduces Fehling's solution.

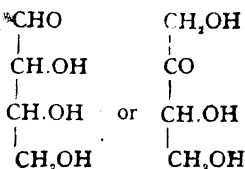
II. Trioses, $C_3H_4O_3$



e.g., **Glyceric aldehyde** :

$\begin{array}{c} \text{CHO} \\ | \\ \text{CH.OH} \\ | \\ \text{CH}_2\text{OH} \end{array}$ The dl-compound has been prepared synthetically; a crystalline substance, m.p. 138° , with a sweet taste; easily soluble in water but less so in organic solvents; reduces Fehling's solution.

III. Tetroses, $C_4H_6O_4$

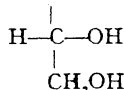


e.g., Erythrose,

CHO	This has been prepared synthetically as a colourless
	sweet syrup, easily soluble in water. It reduces Fehling's
CH.OH	solution. It contains 2 asymmetric carbon atoms and
	both the dextro- and lævo forms have been synthesized;
CH.OH	<i>d</i> -erythrose is lævorotatory and <i>l</i> -erythrose is dextro-
	rotatory (see below), the value for the latter being
CH ₂ OH	$[\alpha]_D^{20} = +21.5^\circ$ (water).

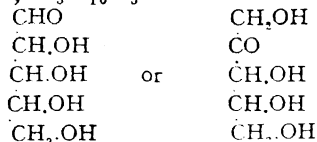
The alcohol corresponding to erythrose, known as *erythritol* or *erythrite*, CH₂OH(CHOH)₂.CH₂OH is found to occur in nature in some algae (e.g. *Protococcus vulgaris*, *Trentepohlia jolithus*, etc.).

N.B. The prefix *d*- or *l*- added to an organic compound should strictly speaking be applied to dextro- and lævo-compounds respectively. In the case of sugars, however, an old convention introduced by Emil Fischer has upset this rule and *d*- or *l*- applied to these show only their genetic relationship to an arbitrarily fixed formula for *dextro-glycerose* from which all the monosaccharides can be synthesized, and not the nature of their optical activity. Thus dextro-glycerose or *d*-glycerose has been assigned the formula



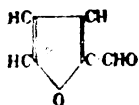
which the OH attached to the asymmetric C atom is written on the right hand side and all the monosaccharides derived from *d*-glycerose are written with the prefix *d*- irrespective of their sign of rotation, and all the monosaccharides derived from *l*-glycerose are written with the prefix *l*-. As a result, lævo-rotatory erythrose is written as *d*-erythrose, dextro-rotatory arabinose is written as *l*-arabinose, lævorotatory fructose is written as *d*-fructose, and so on.

IV. Pentoses, C₅H₁₀O₅.



From the number of asymmetric carbon atoms present, four isomers of the *aldopentoses* (i.e., pentoses with CHO groups) are possible (arabinose, xylose, ribose and lyxose), each with its dextro-, lævo- and inactive forms. Of these, the first two occur widely in nature in the plant kingdom. Ribose has been found in small quantities in yeast nucleic acid whereas lyxose has been obtained only synthetically. A pentose is occasionally found in human urine in a rare condition known as *pentosuria* and this has been shown to be *l*-xyloketose.

The pentoses show the characteristic reactions of the monosaccharides, e.g., reduction of ammoniacal silver nitrate, Fehling's solution, etc., formation of osazones, hydrazones, cyanhydrins, oximes, etc., as well as esters with acids. The aldopentoses are not fermentable with yeast but they are decomposed by certain bacteria yielding acetic, lactic, and other acids.

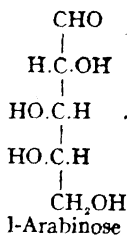


Furfural

A characteristic reaction of the pentoses which differentiates them from hexoses is the formation of *furfuraldehyde*, *furfurole* or *furfural*.

(1) *Furfural reaction*. When a pentose is boiled with concentrated hydrochloric acid it yields furfural which is an aldehyde with a ring structure, volatile with steam. The presence of furfural can be detected by the bright cherry-red colour which it gives when a piece of filter paper moistened with a solution of aniline acetate is held in the distilling vapour. This test is also given by pentosans, and even the amount of pentose or pentosan can be estimated from the furfuraldehyde formed. Pentoses also give the following tests; (2) *Bial's Orcinal Test*; Bial's reagent consists of orcin, conc. HCl and a little dilute ferric chloride. The reagent is heated to boiling and a few drops of the pentose solution added; a green colour is developed. (3) *Phloroglucinol Test*. On heating a mixture of equal volumes of conc. HCl and the pentose solution with a little phloroglucinol, a cherry red colour is developed and a precipitate is formed. The precipitate is soluble in amyl alcohol and this solution shows an absorption band between D and E.

Arabinose, $C_5H_{10}O_5$



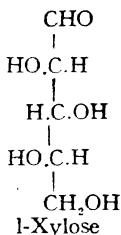
Occurrence. Arabinose does not occur in the free state in nature but is easily obtained by the hydrolysis of the polysaccharide *araban* ($C_5H_8O_4$)_n (see *Araban*). Arabinose can also be obtained by the hydrolysis of some plant mucilages, pectins and glucosides.

Preparation.—Cherry gum or gum arabic is hydrolyzed with 4 per cent sulphuric acid, neutralized with pure calcium carbonate, filtered from calcium sulphate, fermented with yeast to remove the hexoses, and evaporated to a syrup under reduced pressure; the syrup is treated with 4 to 5 volumes of hot 96 per cent alcohol to precipitate the impurities and again concentrated to a syrup. On seeding with pure arabinose crystals separate out.

Properties. l-Arabinose, the sugar obtained from natural sources, crystallizes in colourless needles, m.p. 160° ; it is easily soluble in water but almost insoluble in absolute alcohol and ether; it shows mutarotation and is dextro-rotatory (see note on p. 197) the specific rotation $[\alpha]_D^{20}$ of l-arabinose being $+104.5^{\circ}$ (water); the m.p. of the phenylosazone is 165° . It reduces Fehling's solution. It is not fermented by yeast but some bacteria decompose it and produce lactic, acetic, formic, succinic and oxalic acids. It is used in bacteriological work.

Xylose, Wood Sugar. $C_5H_{10}O_5$,

Occurrence. Xylose does not occur in nature in the free state but is easily obtained by the hydrolysis of the polysaccharide *xylan* or wood gum $(C_5H_8O_4)_n$ (see Xylan) found in cereal straws and grasses, in the wood of deciduous trees, in bark, roots, bran of seeds and grains, etc., and is stated to be the most abundant component of plant constituents next to cellulose.



Preparation. Xylan is heated with dilute HCl, the solution treated with pure lead carbonate until neutral to congo red paper and filtered. The filtrate is evaporated to a thin syrup, treated with strong alcohol to remove gums, lead chloride, etc., the lead removed by sulphuretted hydrogen, filtered and concentrated to a syrup. On cooling, crystals of xylose separate out which are recrystallized from alcohol using some animal charcoal.

Properties. The l-xylose obtained from natural sources crystallizes in colourless needles, m.p. 145° . It has a sweetish taste and is easily soluble in water and hot alcohol. l-xylose shows strong mutarotation, $[\alpha]_D$ five minutes after solution = $+85.68^{\circ}$ (in water) and constant = $+18.5^{\circ}$. It is not fermented by yeast but some bacteria and moulds decompose it to produce lactic, succinic, acetic and l-xylic acids.

Methyl Pentoses

Rhamnose, Isodulcitol, $CH_2.(CHOH)_4.CHO$; it is found as a component of glucosides, such as quercitrin (from the bark of *Quercus citrina*), frangulin (from the bark of *Rhamnus frangula*), hesperidin, etc., from which rhamnose can be obtained by hydrolysis. Rhamnose forms colourless crystals with a sweetish taste and a slight bitter after-taste; $[\alpha]_D^{20}$ for $C_6H_{12}O_5.H_2O = +8.5^{\circ}$ (water); it shows mutarotation. Rhamnose is not fermented by yeast but is decomposed by certain bacteria forming acetic, lactic and other acids. It is used in bacteriological work.

Hexoses, $C_6H_{12}O_6$.

CHO	CH_2OH
CHOH	CO
(CHOH) ₃	(CHOH) ₃
CH_2OH	CH_2OH
Aldohexose	Ketohexose

Like other monosaccharides, the hexoses can be divided into two main groups: (1) *aldoses* or sugars with an aldehyde group, *e.g.*, glucose, and (2) *ketoses* or sugars with a ketone group, *e.g.*, fructose. Of the aldoses, only three occur naturally, *e.g.*, d-glucose, d- and l-galactose and d-mannose, and these are important from the medical point of view. Of the ketoses, only l-fructose and d-sorbose have been found to occur naturally.

Besides the CHO or CO group, a hexose contains some *alcoholic hydroxyl* groups (one primary and 4 secondary in an aldohexose). Many of the characteristic reactions of the sugars may be expected from the presence of these groups (see graphic formula for glucose). Thus the reduction of an ammoniacal solution of silver nitrate, the reduction of Fehling's solution, the oxidation to the corresponding acids, the reduction to corresponding alcohols and the formation of compounds with hydrocyanic acid, hydroxylamine or phenylhydrazine, are all characteristic of the aldehyde group, and the formation of esters, like acetates, nitrates, etc., show the presence of alcoholic hydroxyl groups.

The aldohexoses contain four asymmetric carbon atoms and one would, therefore, expect 16 isomers (including the d- and l-forms) and all of these have been prepared either from natural sources or synthetically.

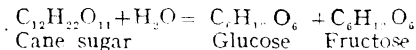
The hexoses are colourless, crystalline substances with a sweet taste. They are easily soluble in water, soluble in dilute alcohol but almost insoluble in absolute alcohol, ether or chloroform. They are fermentable with yeast.

Glucose. *Dextrose, Grape sugar, C₆H₁₂O₆*

$ \begin{array}{c} \text{CHO} \\ \\ \text{H.C.OH} \\ \\ \text{HO.C.H} \\ \\ \text{H.C.OH} \\ \\ \text{H.C.OH} \\ \\ \text{CH}_2\text{OH} \end{array} $	<p><i>Occurrence.</i> The name glucose is derived from the Greek, <i>glukus</i>-sweet. The term <i>grape sugar</i> is derived from the fact that it occurs in fair quantities in ripe grapes. Glucose is found in the <i>free state</i> in almost all ripe fruits as well as in the nectar of flowers. In honey, it occurs from 33 to 40 per cent along with the same quantity of fructose as Invert Sugar (see later). Glucose is found in normal human <i>blood</i> to the extent of about 0.12 per cent and in the blood of diabetic patients the concentration may rise to as high as 0.4 per cent. Normal human urine contains less than 0.1 per cent of glucose but in diabetes the content rises up to 6 or 7 per cent or even higher. <i>In combination</i>, glucose is found in disaccharides, trisaccharides, etc., in glucosides, and in polysaccharides such as starch, glycogen, cellulose, etc.</p>
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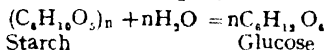
Preparation.

In the laboratory, glucose is most easily prepared by the hydrolysis of cane sugar. Powdered cane sugar (50 g.), is dissolved in 90 per cent alcohol (150 c.c.), some concentrated HCl (6 c.c.) added as a hydrolyzing agent, the solution is warmed to about 50° and allowed to remain at this temperature for about 2 hours, it is then transferred to an ice chest and seeded with a few crystals of glucose. After a few days the crystalline glucose which separates out can be filtered off, the more soluble fructose formed along with glucose remaining in solution. The glucose is washed with alcohol and purified by recrystallization from dilute alcohol (2:1). The reaction is as follows:



On a large scale, glucose is prepared by the hydrolysis of starch obtained from potato, rice or maize. The starch is heated with about 0.5 per cent sulphuric acid at a temperature of about 125° and a pressure of 2 to 3 atmospheres. After hydrolysis is complete, the sulphuric acid is neutralized with chalk and the calcium sulphate removed by filtration.

The filtrate is decolorized with animal charcoal and then concentrated to a syrup *in vacuo*. The glucose crystallizes out and is further purified by recrystallization from dilute alcohol. It is important to remember that *commercial glucose* may contain traces of *arsenic* or *lead* derived from the impure sulphuric acid used in its preparation; commercial glucose may also contain some *dextrin* and *maltose* derived from starch. The reaction in this preparation is as follows:



Physical Properties of Glucose.—Glucose crystallizes from water at room temperature as the *hydrate* $C_6H_{12}O_6 \cdot H_2O$ in plates, m.p. 86° , whereas the anhydrous substance $C_6H_{12}O_6$ crystallizes from ethyl or methyl alcohol in colourless needles, m.p. 146° . Glucose is about half as sweet as cane sugar. It is dextrorotatory and is hence known as *dextrose*; $[\alpha]_D = +52.5^\circ$ (in water); it shows mutarotation (see p. 208). It is easily soluble in water, 100 parts of water dissolving 81.68 parts of anhydrous glucose at 17.5° . Glucose is almost insoluble in absolute alcohol but the solubility increases with dilution, 100 parts of 89 per cent alcohol dissolving 1.94 parts of anhydrous glucose at 17.5° and 21.7 parts at the boiling point. It is partially soluble in methyl alcohol but almost insoluble in ether, ethyl acetate or acetone. A saturated solution of glucose in pyridine contains 7.62 parts of glucose in 100 parts of the solution at 26° .

Uses.—The most important use of glucose is as food. Practically all digestible carbohydrates are converted by various ferments in the body to glucose, and are absorbed into the body as such. After a meal rich in carbohydrate, the blood sugar rapidly rises and remains high for about two hours. Commercial glucose is largely employed in the preparation of confectionery, jams, etc., in the preservation of fruits, and in silvering mirrors. Solutions of pure glucose and of calcium gluconate, prepared from glucose, are used widely in medicine either subcutaneously or intravenously.

Reactions and Tests for Glucose.

1. **Action of Heat.**—On heating, glucose first melts and on further heating it turns brown giving off a smell resembling burnt sugar.

2. **Action of Acids.**—Concentrated sulphuric acid does not char glucose in the cold and it is thus distinguished from cane sugar; charring takes place after heating for some time. Prolonged heating with dilute sulphuric acid or hydrochloric acid will produce humus-like substances.

3. **Action of Alkalies.**—A dilute solution of *sodium carbonate* or *ammonia* does not produce much change but solutions of *caustic alkalies* produce profound changes, especially when warmed. When heated with caustic soda, the solution first becomes yellow, reddish brown and then dark brown (dienol reaction); this is sometimes used as a test for reducing sugars and is known as *Moore's Test*. If a solution of glucose is treated with *lime water* followed by alcohol, *calcium glucosate* $C_6H_{12}O_6 \cdot CaO$ is precipitated; it is soluble in water but insoluble in alcohol and is decomposed by CO_2 . A similar compound with baryta is known.

4. **Nitro-chromic Reaction.**—To about 3 c.c. of the solution are added 5 c.c. of conc. HNO_3 and 5 drops of 5 per cent solution of potassium chromate. Mixed well. A blue colour develops in about a minute. All sugars including cane sugar give this reaction.

The test depends on the presence of $-CH.OH$ groups and is therefore given by all primary and secondary alcohols (including glycerol), formaldehyde, lactic acid, hydroxy-butyric acid, &c., but not by polysaccharides.

5. **Dinitrobenzene Reaction.**—To about 4 c.c. of the solution made alkaline with 5-10 drops of 20 per cent $NaOH$ solution, are added 4 drops of 5 per cent alcoholic solution of o-dinitrobenzene. Mixed and warmed gently. A violet colour develops. All reducing sugars give this test which is due to dienolisation of sugar. Shaking inhibits the reaction.

6. **Reducing Action.**—Owing to the presence of free or potential aldehyde group, glucose acts as a good reducing agent, the CHO group being itself oxidized to $COOH$. Thus it reduces alkaline solutions of the salts of silver, bismuth or copper either to the metals or to their lower oxides: *e.g.*,

(1) **Silver.**—Ammonia is added to a solution of $AgNO_3$ until the precipitate formed just redissolves; on adding a solution of glucose and warming the mixture in the water bath a mirror of silver is formed.

(2) **Bismuth.**—A solution of bismuth subnitrate and Rochelle salt in 8 per cent $NaOH$, known as *Nylander's solution*, is reduced when boiled with a solution of glucose giving a black precipitate of metallic bismuth. Nylander's solution is not reduced by creatinine or uric acid and is thus useful in the detection of glucose in urine.

(3) *Copper.*

(a) *Barfoed's Reagent.*—If a dilute solution of cupric acetate in glacial acetic acid, known as Barfoed's reagent, is boiled with glucose solution, a precipitate of red cuprous oxide (Cu_2O) is obtained. This reaction is given by glucose and other monosaccharides but not by maltose and lactose (disaccharides).

(b) *Fehling's Solution.*—When a solution of glucose is boiled with a solution containing copper sulphate, Rochelle salt and caustic soda, known as Fehling's solution, there is a precipitate of red cuprous oxide (Cu_2O) formed by the reduction of the cupric sulphate of the solution.

Fehling's solution is also used for the *quantitative estimation* of glucose—ten cubic centimetres of Fehling's solution being equivalent to 0.05 gram of pure glucose. To carry out an estimation, 10 c.c. of Fehling's solution are taken in a small conical flask, diluted with some water and kept boiling over an wire-gauze. The sugar solution is gradually added from a burette until the blue colour just disappears, the end point being judged by testing a drop with some convenient indicator such as a solution of potassium ferrocyanide acidified with acetic acid, or a solution of starch and KI. The use of an internal indicator, such as a 1 per cent solution of *methylene blue* added to the boiling mixture near the end of the reaction, is also found to be convenient and accurate. The discharge of the blue colour of methylene blue in the boiling mixture indicates the end point.

The Fehling's solution used in the test consists of two solutions which are kept in two separate bottles, a mixture of the two in equal volumes being the final solution used in qualitative or quantitative tests. The first solution or No. I contains 69.28 grams of pure copper sulphate crystals ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) per litre of water. The second solution or No. II contains 346 grams of Rochelle salt (sodium potassium tartrate) and 130 grams of sodium hydroxide per litre of water. The Rochelle salt prevents the precipitation of cupric hydrate formed by the action of caustic soda (see tartaric acid, p. 190).

The use of Fehling's solution is attended with some *objections*: (i) the NaOH present may destroy some of the sugar, (ii) the end point is not determined easily, (iii) the mixed reagent not being stable, it has to be kept in two separate bottles and the final solution has to be tested before use, (iv) it is also reduced by creatinine, urates, glycuronates, and a few other constituents of urine. In spite of these drawbacks, Fehling's solution is widely used in the estimation of reducing sugars. In biochemical work, however, it is displaced in many cases by Benedict's solution.

(c) **Benedict's Solution.**—It is really a modification of Fehling's solution, the strong alkali NaOH being replaced by sodium carbonate and tartrate replaced by citrate. Benedict's solution is not easily reduced by urates, etc., and it is thus superior to Fehling's solution for the detection of glucose in urine.

Two solutions are used, the one for *qualitative tests* containing 17.3 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 173 grams of sodium citrate and 100 grams of anhydrous sodium carbonate per litre of water. The solution, which is used for *quantitative estimations*, is made as follows: 100 grams of anhydrous sodium carbonate or its equivalent weight of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, 200 grams of sodium citrate and 125 grams of potassium thiocyanate are dissolved in hot water, made to about 800 c.c. and filtered. 18 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ are dissolved in 100 c.c. of water and added to the above solution, 5 c.c. of a 5 per cent solution of potassium ferrocyanide are then added to the mixture and the whole made up to one litre. 25 c.c. of this solution are equivalent to 0.05 gram of glucose.

To carry out an estimation, 25 c.c. of Benedict's solution are taken in a conical flask, about 5 grams of Na_2CO_3 are added and the solution kept boiling over an wire gauze. The sugar solution is run in from a burette until the blue colour is discharged and milky white colour and precipitate appear. To increase the sensitiveness and to enable the point to be judged correctly, a drop or two of a one per cent solution of methylene blue is added towards the end. The copper in Benedict's solution is reduced to cuprous thiocyanate which comes down as a heavy white precipitate being insoluble in sodium carbonate.

7. Action of Phenylhydrazines.

(d) *Formation of phenylhydrazones.*—Owing to the presence of the aldehyde group (see aldehydes), glucose combines with phenylhydrazine, or a substituted phenylhydrazine, when treated with the reagent in the cold in presence of dilute acetic acid, giving a phenylhydrazone; these hydrazones sometimes serve to identify a sugar by their characteristic melting points, e.g., *glucosephenylhydrazone* $\text{CH}_2\text{OH}(\text{CH}(\text{OH}))_4\text{CH}:\text{N}(\text{NH}(\text{C}_6\text{H}_5))_2$ forms two isomers, m.p. 115° and 144° ; *glucosediphenylhydrazone* $\text{CH}_2\text{OH}(\text{CH}(\text{OH}))_4\text{CH}:\text{N}(\text{N}(\text{C}_6\text{H}_5))_2$, colourless prisms, m.p. 161° .

(b) *Formation of Osazones.*—When, however, glucose is heated with a solution of phenylhydrazine in acetic acid, it forms a yellow crystalline substance called *phenylglucosazone*. These osazones are given by many reducing sugars and the crystalline structure, solubility and melting point of the osazone produced (also with different substituted phenylhydrazines) help us in the identification of many sugars.

Phenylhydrazine is mixed with an equal volume of glacial acetic acid, diluted with some water and heated with the solution of glucose in a boiling water bath for about 10 minutes, when crystals of phenylglucosazone separate out and can be purified by recrystallization from dilute alcohol. It forms long yellow needles mostly in

sheafs, m.p. 204-205° (Fig. 34). The reaction takes place as follows: the glucose phenylhydrazone first formed is oxidized to a ketone by a second molecule of phenylhydrazine and the ketone unites with a third molecule of phenylhydrazine to form the osazone:

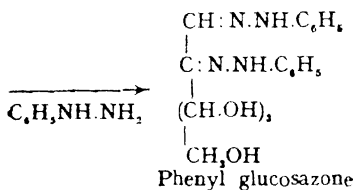
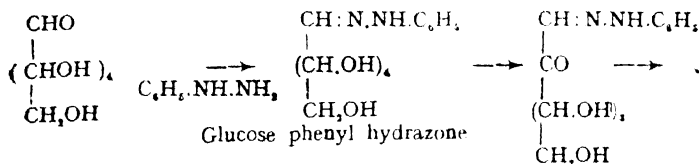
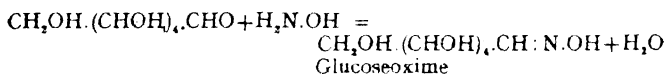
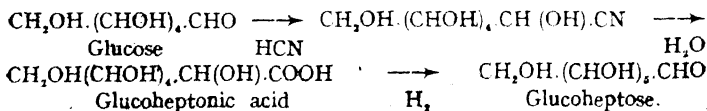


FIG. 34.

8. **Action of Hydroxylamine :** With hydroxylamine glucose gives glucoseoxime.

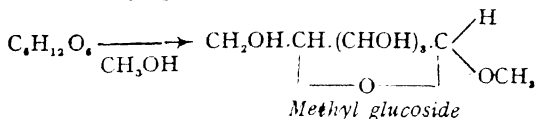


9. **Action of Hydrocyanic Acid :** With HCN glucose gives a cyanhydrin; on hydrolysis, this cyanhydrin yields an acid which on reduction gives the "higher" monosaccharide, glucoheptose; these reactions thus enable one to build up a higher mono-accharide from a lower one.



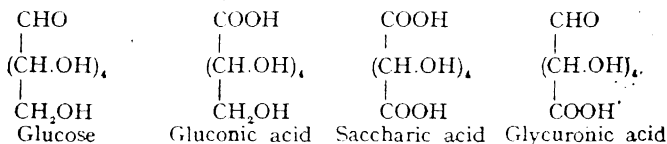
10. **Action of Acid Radicals, Formation of Esters.**—With acetic anhydride glucose gives pentacetylglucose or *glucose pentaacetate* $\text{CH}_3\text{.O.COCH}_2\text{.}(\text{CH.O.COCH}_2)_4\text{.CHO}$; with benzoyl chloride it gives *glucosepentabenzoate* $\text{CH}_3\text{.O.COC}_6\text{H}_5\text{.}(\text{CH.O.COC}_6\text{H}_5)_4\text{.CHO}$; all these reactions show the presence of five alcoholic hydroxyl groups.

11. **Action of Alcohols, Formation of Glucosides.**—When dry HCl gas is passed into a solution of glucose in dry methyl alcohol, α - and β - methyl glucosides are obtained (see later):



12. **Action of Reducing Agents.** When reduced with sodium amalgam, glucose is reduced to sorbitol, the CHO group being converted into CH_2OH . *d*-Sorbitol or *sorbitol* occurs naturally in sorb apples, mountain-ash berries, and in some other fruits. It is used in bacteriological work.

13. **Action of Oxidizing Agents.** With mild oxidizing agents such as bromine, the CHO group is oxidized to COOH giving *gluconic acid*. The reaction takes place smoothly when one part of the sugar is treated with 5 parts of water and 2 parts of bromine and the solution kept at room temperature for 1 to 3 days. Gluconic acid is used largely in medicine as *calcium gluconate* for the administration of calcium. With more powerful oxidizing agents, such as nitric acid, the primary alcohol group of glucose is also oxidized and *saccharic acid* is formed. Another oxidation product of glucose is *glycuronic acid*, in which the CHO group remains unchanged but the terminal CH_2OH group is oxidized to COOH. It is excreted as glucuronates in the urine but the amount is very much increased if toxic substances, such as chloral, camphor, morphine, phenol, antipyrine, etc., are administered, these substances being eliminated in combination with glycuronic acid.



14. Fermentation.

Glucose is fermented or decomposed by a number of fungi and bacteria, and the nature of the main product formed depends upon the nature of the organism selected. A few of the important fermentations, used in the manufacture of organic compounds, may be mentioned here:

(a) *Alcoholic Fermentation*—Glucose is fermented by certain species of yeast, such as *Saccharomyces cerevisiae* or brewer's yeast, producing ethyl alcohol and carbon dioxide (see ethyl alcohol). Since glucose is the only fermentable sugar found in urine, it can be estimated from the amount of CO_2 produced by the action of yeast.

(b) *Lactic Acid Fermentation*.—See lactic acid (p. 180).

(c) *Butyl Alcohol-Acetone Fermentation*.—See butyl alcohol (p. 105).

(d) *Butyric Acid Fermentation*.—See butyric acid (p. 143).

(e) *Oxalic Acid Fermentation*.—Glucose is fermented into oxalic acid by a large number of moulds and bacteria.

Estimation of Glucose in Blood.—The method usually followed in biochemical work is a modification of Folin's method. 0.2 c.c. of blood is taken in a small test tube and 3.2 c.c. of distilled water is added to lake it. The proteins are then precipitated by adding 0.3 c.c. of a 10 per cent solution of sodium tungstate, followed by 0.3 c.c. of a 2/3 normal H_2SO_4 . It is then filtered and 2 c.c. of the filtrate (corresponding to 0.1 c.c. of blood) are taken and heated with 2 c.c. of an alkaline solution of copper sulphate (containing 4.5 g. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 7.5 g. tartaric acid and 40 g. anhydrous sodium carbonate per litre). The solution is then treated with 2 c.c. of a solution of phosphomolybdic acid. A blue colour is developed with the cuprous oxide formed, whereas the blue colour of the excess of copper sulphate is discharged. The amount of sugar is then calculated by matching the blue colour against that produced by a standard solution (0.02 per cent) of glucose in a colorimeter.

N.B.—Instead of adding 0.3 c.c. of 2/3 normal H_2SO_4 to the tungstate mixture, 0.3 c.c. of 7% copper sulphate soln. may be added.

Mutarotation and Different Forms of Glucose.

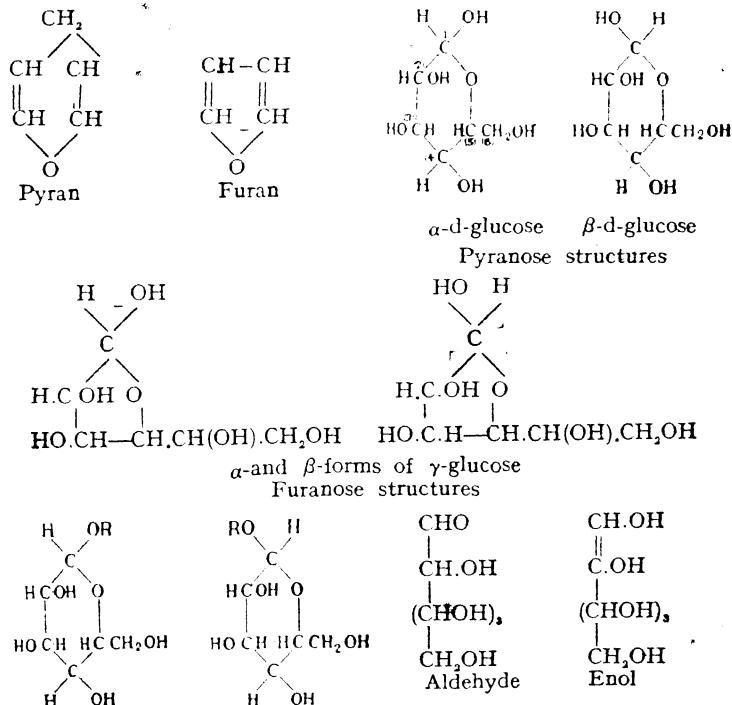
The different stereoisomers of glucose have been based on the assumption that they possess a simple aldehydic structure. Several facts, however, lead us to believe that the structure of each of these sugars is of a more complex nature.

Glucose, for instance, does not show many of the characteristic reactions of an aldehyde: it does not give Schiff's reaction, it does not readily react with sodium hydrogen sulphite nor even with phenylhydrazine-p-sulphonic acid. Again, ordinary glucose shows a specific rotation of $+111^\circ$ immediately after solution. The rotation gradually decreases and finally becomes constant at $+52.5^\circ$. This phenomenon of a change of rotation from an initial value to a constant one is called *mutarotation*. If the ordinary glucose is crystallized from pyridine, another modification is obtained which has an initial rotation of $+17.5^\circ$; the rotation gradually increases and finally becomes constant at $+52.5^\circ$. There are thus two forms of d-glucose, one with a high initial rotation and another with a low initial rotation. In aqueous solution each form is supposed to be transformed into the other until an equilibrated mixture is obtained having the rotation of $+52.5^\circ$. This equilibrium is attained almost instantaneously by adding a drop of dilute alkali like ammonia to a solution of either form of glucose.

Again, glucose combines with methyl alcohol as mentioned before, and gives compounds known as *methyl glucosides*. These compounds do not behave as aldehydes and are found to occur in two isomeric forms, α - and β -methyl glucosides. It has also been found that α -methyl glucoside is hydrolyzed to glucose with a high

initial rotation and β -methyl glucoside is hydrolyzed to glucose with a low initial rotation. The form of glucose with a high initial rotation is thus called α -glucose and the one with low initial rotation is called β -glucose. In equilibrium in aqueous solution, the α - and β -forms of d-glucose are stated to be present in the proportion of 1:2. The presence of the α - and β -forms also shows that glucose has five asymmetric carbon atoms.

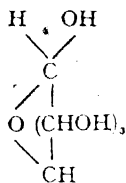
Recent researches have shown that α - and β -forms of d-glucose have the *pyranose* ring structure. Another form of d-glucose, known as γ -glucose is supposed to have a *furanose* ring structure and it is interesting to note that the β -form of γ -glucose is strongly laevorotatory. d-Glucose may, therefore, exist in pyranose, furanose, aldehyde and enol forms, and the structure of these, according to Haworth and others, are shown below; the structures of α - and β -methyl glucosides are also shown here for comparison:



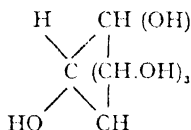
α -methyl glucoside. β -methyl glucoside.

Mechanism of Mutarotation. It was first suggested by Armstrong that the change from α - to β -form or *vice versa* was an

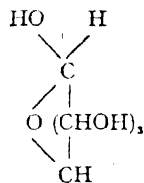
isomeric change brought about by the addition of water, probably with the formation of an *oxonium hydrate* (having a tetravalent oxygen atom) as an intermediate product: e.g.,



CH_2OH
 α -glucose



CH_2OH
Intermediate product

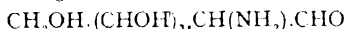


CH_2OH
 β -glucose

Mackenzie and Ghosh (Proc. Roy. Soc. Edin. 1914-15) have, however, shown by a study of the mutarotation of different sugars in pure *formamide* that the presence of water is not essential, and although water may act in the manner suggested by Armstrong in aqueous solutions, the mutarotation in non-aqueous solvents can be explained by a similar combination of the solvent with the sugar.

Chitin. This is a nitrogenous polysaccharide found in the exoskeletons of insects and crustacea, e.g., shells of lobsters, and in certain fungi. Boiling with conc. HCl splits it into glucosamine and other products.

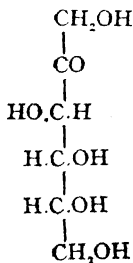
Glucosamine or *Chitosamine*.—Obtained by the hydrolysis of chitin and is 2-amino-glucose of the formula,



It is an important constituent of mucins, etc., (proteins found in mucous secretions or mucus). It reduces Fehling's solution. Normal urine containing excess of mucus may, therefore, slightly reduce Fehling's solution and thus be mistaken for glycosuria.

Chondrosamine is another amino-sugar (2-amino-galactose) obtained as a hydrolytic product of chondro mucoid, a mucoprotein found in cartilage, tendon, etc., chondroitin being an intermediate hydrolytic product.

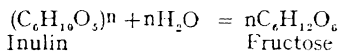
Fructose, *Lævulose*, *Fruit Sugar*, *d-Fructose*, $\text{C}_6\text{H}_{12}\text{O}_6$



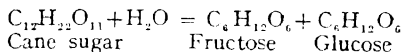
d-Fructose

Occurrence.—In the *free state* it occurs together with glucose in honey (see glucose) and in many sweet fruits, whence the name 'fruit sugar'. It is *also* found in the sap of palmyra palm. *In combination*, it is found along with glucose in cane sugar and raffinose, and in inulin (see), a polysaccharide which is composed of fructose units only.

Preparation.—(a) *From Inulin*: On a large scale, inulin is hydrolyzed by boiling with dilute H_2SO_4 (about 0.5 per cent) for about an hour, the acid is removed by BaCO_3 , the solution decolorized with animal charcoal and filtered. The filtrate is concentrated in vacuo to a thin syrup and finally taken up with alcohol from which it is allowed to crystallize. It can be purified further by crystallization from hot absolute alcohol.



(b) *From Cane Sugar*: In the laboratory, it can be prepared by hydrolyzing cane sugar. A 10 per cent aqueous solution of cane sugar (100 g.) is heated with conc. HCl (2 c.c.) at 60° . The hydrolyzed solution is cooled to -5° and treated with freshly powdered pure calcium hydroxide (60 g.) with constant stirring and filtered rapidly. The filtrate which is kept cold soon deposits fine crystals of calcium fructosate $\text{C}_6\text{H}_{12}\text{O}_6 \cdot \text{CaO}$. After 24 hours, the crystals are filtered off and washed with ice-water. They are then suspended in water at 20° and decomposed with the calculated quantity of oxalic acid. The filtrate is evaporated in vacuo to a syrup at a low temperature, the syrup dissolved in warm absolute alcohol, decanted from the insoluble portion and the alcoholic extract allowed to stand, when crystals of d-fructose separate out.



Properties and Reactions.—d-Fructose crystallizes from alcohol in colourless prisms in the anhydrous state, m.p. 95° . It is more than twice as sweet as glucose, and is stated to be the sweetest of all sugars.

N.B. It has been stated by Cameron that taking the sweetness of cane sugar as 100, the following figures represent the *relative sweetness* of some of the common sugars:

Fructose 173.3, invert sugar 127.4, cane sugar 100.0, glucose 74.3, xylose 40.0, maltose 32.5, rhamnose 32.5, galactose 32.1, raffinose 22.6, and lactose 16.0.

It is very easily soluble in water; it is more easily soluble in alcohol than glucose, one part dissolving in 11.8 parts of

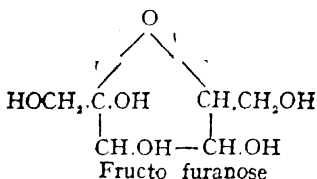
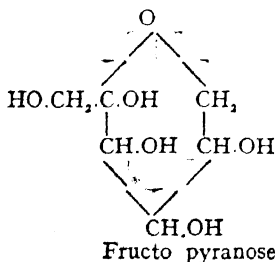
absolute alcohol at 18° . It is strongly lævorotatory and is therefore called 'lævulose', the name d-fructose being retained owing to its generic relationship to d-glucose (see p. 197). Like glucose, it shows mutarotation, the initial rotation for ordinary fructose (β -form) being $[\alpha]_D = -133.5^{\circ}$, and the final constant rotation $[\alpha]_D = -92^{\circ}$. The calculated rotation of the α -form $[\alpha]_D = -21^{\circ}$.

As is evident from the formula, it is a *keto-hexose*, having a ketone group (CO) instead of the aldehyde group. Like glucose it strongly reduces Fehling's solution. With phenylhydrazine, it forms an osazone identical with phenylglucosazone, showing identity of structure of all the uncombined groups of the two sugars. With methylphenylhydrazine, fructose gives a characteristic *methylphenyl-osazone, not given by glucose*, having a melting point 160° . Fructose is easily fermented by yeast. As mentioned in its preparation, fructose forms a lime compound, calcium fructosate, which is less soluble in water than the corresponding glucose compound, and which is decomposed by CO_2 .

A characteristic test for fructose (also given by other keto-hexoses and by cane sugar which yields fructose on hydrolysis) is the *Selivanoff's Test*, which is carried out as follows: To a solution of fructose mixed with an equal volume of conc. HCl, a few crystals of resorcin are added and the solution warmed; a deep red colour is formed and a brown-red precipitate is finally obtained. The precipitate dissolves in amyl alcohol giving a red colour. The reaction is due to the formation of *w*-hydroxymethylfurfural.

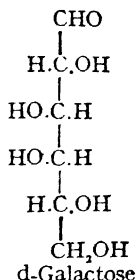
Simply by boiling equal amounts of fructose or cane sugar solution and conc. HCl, an orange colouration is obtained—this is known as **Ketose Test**.

Other structures of Fructose:—

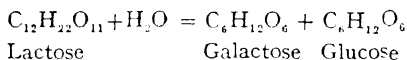


Galactose, *d*-Galactose, $C_6H_{12}O_6$.

Occurrence.—It does not generally occur in the free state in nature but it is widely distributed in a combined state. Thus it is found in combination with glucose in the lactose of milk, in lipoids known as cerebrosides (found in nerve and brain tissues), and as a component of vegetable gums, pectins and mucilages. Agar-agar, a mucilage obtained from certain seaweeds, contains a high percentage of galactose.



Preparation.—Lactose (50 g.) is heated with 2 per cent sulphuric acid (500 c.c.) over a boiling water bath for about 4 hours. The free acid is neutralized with an excess of BaCO_3 and the solution filtered. The filtrate is evaporated to a syrup and inoculated with a few crystals of pure galactose. The crystals which are obtained are filtered off, washed with a little 80 per cent alcohol and redissolved in the smallest amount of hot water. Hot strong alcohol is added, the solution boiled with animal charcoal and filtered. On cooling, pure crystals of galactose separate out.



Properties and Reactions.—*d*-Galactose crystallizes from alcohol in colourless, minute hexagonal crystals, m.p. 165.5° . It is less sweet than cane sugar. It is easily soluble in hot water, moderately soluble in 50 per cent alcohol but practically insoluble in absolute alcohol. It shows mutarotation, the final constant rotation being $[\alpha]_D^{20} = +81.5^\circ$; the $[\alpha]_D$ for α -galactose is stated to be $+145^\circ$ and that for β -galactose $+54^\circ$. With phenylhydrazine, it forms galactose phenylosazone, m.p. 196° . It is fermented slowly and only by some yeasts. Like glucose, galactose forms *galacturonic acid* by the oxidation of the primary alcohol group. Galacturonic and other *uronic acids* are found in some natural gums, pectins, etc. On oxidation with nitric acid, galactose yields *mucic acid*, $\text{HOOC}(\text{CH}_2\text{OH})_4\text{COOH}$, a dibasic acid isomeric with saccharic acid. Mucic acid crystallizes in granular prisms, m.p. $212-215^\circ$, and is almost insoluble in water. The formation of mucic acid is utilized in identifying as well as in estimating the amount of galactose present. On reduction, galactose gives *dulcitol* or *dulcite*, an inactive alcohol occurring in Madagascar manna (*Melampyrum nemorosum* L.) and other plants. Dulcite is used in bacteriological work.

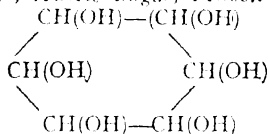
Mannose, d-Mannose, $C_6H_{12}O_6$

CHO
|
OH.C.H.
|
OH.C.H.
|
H.C.OH
|
H.C.OH

Occurrence.—Mannose is not found in the free state in nature but it is widely distributed in plants as the polysaccharide *mannan* or *mannosan* ($C_6H_{10}O_5$)_n which belongs to the type of hemicelluloses. Mannan occurs in Salep mucilage, in yeast gum, in ivory nuts, etc. Mannose has also been found in combination in animal substances such as egg albumin, the globulin of the blood serum of horse and ox, in tubercle bacilli, etc.

Preparation.—d-Mannose is best prepared from ivory nuts, i.e., the seeds obtained from Tagua d-Mannose palm, *Phytelephas macrocarpa* R. & P., used in making artificial ivory buttons, etc. The shavings of the nuts from button factories are hydrolyzed by 75 per cent sulphuric acid, the acid neutralized with $CaCO_3$, the filtrate decolorized with bone charcoal, and concentrated to a thin syrup in vacuo. The syrup is treated with 95 per cent alcohol and filtered. The filtrate is concentrated to a thin syrup and treated with an equal volume of glacial acetic acid when crystals of pure mannose separate out.

Properties and Reactions.—d-Mannose crystallizes in colourless prisms, m.p. 132° . It has a pleasant sweet taste; it is readily soluble in water and in 80 per cent alcohol. It shows mutarotation, the final constant rotation being $[\alpha]_D = +14.25^\circ$. It reduces Fehling's solution and forms the same osazone as that given by glucose and fructose. It is easily fermented by yeast. On reduction, it gives *mannitol* or *mannite*, which is found to occur in nature in ash manna, in algae, bacteria, fungi, etc.

Inositol, Inosite, Muscle Sugar, 4-Inositol, $C_6H_{12}O_6$ 

Inositol is inactive and isomeric with the hexoses but strictly speaking it is *not a carbohydrate* at all and can be classed with *polyhydroxyphenols* (see Phenols). It has a slight sweet taste and occurs in muscle (hence known as 'muscle sugar') as well as in other tissues. *Phytin*, a substance which occurs widely in plants, is the calcium and magnesium salt of inositol phosphoric acid which is split up by the specific enzyme phytase into inositol and phosphate.

Properties and Tests.—Inositol crystallizes in colourless prisms, m.p. 225° ; it is very readily soluble in water, less soluble in alcohol and insoluble in ether; it is not fermentable and does not reduce Fehling's solution. When inositol is evaporated almost to dryness with nitric acid and then evaporated carefully with an ammoniacal solution of calcium chloride, a rose red colour is produced (*Scheerer's Test*); with ammoniacal strontium acetate in place of calcium chloride a violet tint is obtained.

CHAPTER XVII

CARBOHYDRATES (*Continued*)

Class II. Compound Sugars, Oligosaccharides.

I. Disaccharides.—As the name suggests, a disaccharide is composed of two monosaccharide units. The common and important ones are sucrose, lactose and maltose, and they are derived from two hexose units, with the elimination of a molecule of water: $C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O$. They are sweet crystalline substances, soluble in water. They are hydrolyzed by dilute mineral acids or by appropriate enzymes to their component hexoses. They are not directly fermentable with yeast but can be fermented after hydrolysis to the monosaccharides. Of the above three disaccharides, lactose and maltose reduce Fehling's solution whereas sucrose is non-reducing, showing the absence of any ketone or aldehyde group in the latter. All the three sugars yield octacetyl derivatives, showing the presence of 8 alcoholic OH groups. *The disaccharides do not reduce Barfoed's reagent.*

Sucrose, Cane Sugar, Beet Sugar, Saccharose, $C_{12}H_{22}O_{11}$.

Occurrence.—Cane sugar is fairly widely distributed in plants. In the juice of the sugar cane and in the juice of the red beet root, it may occur to the extent of about 15 per cent and these are, therefore, cultivated by improved methods for the manufacture of cane sugar. It is also found in large quantities in some species of palm (commonly, date-palm) growing in India from which crude sugar or *gur* is made. It occurs in ripe pine apples (about 10 per cent), in ripe bananas (about 5 per cent), and in several other fruits, in the nectar of flowers, in honey (0 to 5 per cent), etc.

Preparation.

(1) *From Sugar Cane.*—The canes are cut into pieces and passed through hydraulic rollers to express the juice. Milk of lime is added to the expressed juice and the mixture heated; this coagulates the proteins, neutralizes the acids and precipitates

some of the acids as lime salts. The precipitates are removed by filtration and the filtrate treated with carbon dioxide which precipitates any uncombined lime and also decomposes the calcium saccharate formed; it is then treated with sulphur dioxide to decolorize the solution. The juice is then boiled, filtered and evaporated in vacuum pans heated with steam to a suitable consistency for crystallization. The crystals are removed from the non-crystallizable portion by centrifuging. The non-crystallizable mother-liquor is termed *molasses* which is used for various purposes (see below).

To recover some of the cane sugar found in molasses, it is treated with a hot saturated solution of calcium hydrate $\text{Ca}(\text{OH})_2$ which combines with the sucrose present forming *calcium sucrate*, $\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{CaO}$. This is removed by filtration, dissolved in water, and decomposed by the action of carbon dioxide. The precipitated calcium carbonate is filtered off and the filtrate concentrated in vacuo when a further crop of sucrose crystallizes out. The crystals are separated and dried by centrifugal machine. The mother-liquor from this last crystallization, which still contains some fermentable sugars, is utilized for the same purposes as molasses.

The sugar thus prepared is not quite colourless and so it is refined further. It is dissolved in hot water, the solution clarified with lime or other agents and the clarified solution is decolorized by passing through filters of charcoal or bone-black. The colourless solution is concentrated in vacuum to a magma of crystals which are separated by a centrifugal machine and dried. This procedure is not adopted in this country.

(2) *From Beet Root*.—The roots of the red beet, in which the content of cane sugar has been increased by proper selection of seeds and by improved methods of cultivation, are cut into thin slices, placed in a series of tanks and allowed to soak in warm water. During this period of soaking or lixiviation, the diffusible substances (crystalloids) in the vegetable cells diffuse out into the water while the colloids remain in the cells. The tanks are arranged in such a manner that water circulates from one to the other, the incoming fresh water first meeting the most macerated pulp and the outgoing water meeting the freshly cut slices. This outgoing water laden with cane sugar is subsequently treated in the same manner as the juice expressed from the sugar cane (see above).

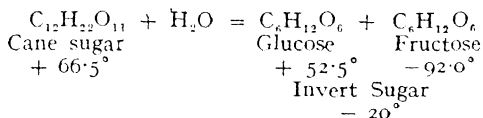
Molasses, obtained either from the sugar cane or from beet roots, contain 50–60 per cent of fermentable sugars. It is sometimes inverted (i.e., hydrolyzed to get invert-sugar), clarified and used as *treacle* or *golden syrup*. Both cane and beet molasses are utilized for manufacturing alcohol, acetone, butyl alcohol, citric acid, lactic acid, etc., by fermentation.

The term molasses is very loosely used. The waste molasses from sugar factory containing non-crystallisable sugar is to be distinguished from ordinary crystallisable molasses or *gur*. Both are used in the distillery for alcoholic fermentation.

Properties, Reactions and Uses.—Cane sugar crystallizes from water in colourless monoclinic prisms *without any water of crystallization*. It is easily soluble in water, dissolving in about **half** its weight of **water** at 20° and is only slightly soluble in alcohol. When heated to 160° it melts to a glassy mass which on cooling forms an amber coloured amorphous solid known as *barley sugar*; on heating further, it becomes dark brown and is then known as caramel, which is used for colouring wines and sweets. Cane sugar is **extensively** used as food—as table sugar and also in confectionery and in cooking.

Cane sugar does not show mutarotation, and its specific rotation $[\alpha]_D^{20} = +66.5^{\circ}$ (water). It does not reduce Fehling's solution but does so after hydrolysis with a dilute acid. It does not react with phenylhydrazine. It gives Selivanoff's test and Ketose Test (see p. 212) owing to the presence of fructose in its molecule. It is fermented by ordinary yeast and this is due to the fact that yeast contains the enzyme *invertase* which first hydrolyzes the sugar to glucose and fructose and these are then fermented by the enzyme *zymase*.

The hydrolysis of cane sugar to glucose and fructose by means of dilute acids or by enzymes is known as *inversion* and the mixture of glucose and fructose thus formed is known as *invert sugar*. The term is derived from the fact that cane sugar is dextrorotatory but after hydrolysis the

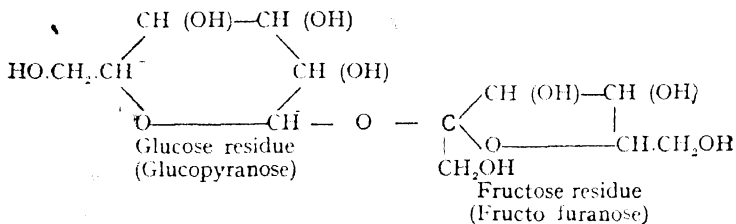


solution becomes lævorotatory, the optical activity of fructose in the lævo direction being greater than the dextrorotation of the glucose fraction. It is interesting to remember that invert sugar is sweeter than cane sugar.

Lime (CaO) is found to be more soluble in a solution of cane sugar than in pure water, and sucrose is known to form compounds like *calcium sucrate* $\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{CaO}$, *strontium sucrate* $\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{SrO}$, etc. These sucates, as mentioned

before, are taken advantage of in the recovery of cane sugar from molasses.

The structure of cane sugar, according to the work of Haworth and others, is as follows:—



Lactose, Milk Sugar, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$

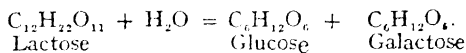
Occurrence.—Lactose occurs in milk of all mammals. In human milk it is present to the extent of 6 to 8 per cent and in cow's milk about 4 per cent. Lactose may occur in the urine of women during lactation. It has not been found in the vegetable kingdom.

Preparation.—Lactose is prepared on a large scale as a bye-product in cheese factories. The milk is coagulated either with the ferment *rennet* or with dilute acetic acid. The *whey*, obtained after filtering off the casein and fat, contains about 4.8 per cent of lactose. It is heated to about 80° and treated with milk of lime which removes certain impurities, filtered, the filtrate saturated with carbon dioxide and concentrated in vacuo. The crystals of lactose are removed by a centrifugal machine, and purified further by recrystallization from water with the help of animal charcoal.

Properties, Uses and Reactions.—Lactose crystallizes from water in colourless, large rhombic crystals with one molecule of water of crystallization $\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_2\text{O}$, which is not lost even on prolonged drying at 100° . It is much less sweeter than glucose (see p. 211). It dissolves in water to the extent of about 17.8 per cent at 25° , but it is insoluble in ethyl or methyl alcohol. It shows mutarotation; the commercial milk sugar is α -lactose showing an initial sp. rotation of $+90^\circ$ and β -lactose shows the initial sp.

rotation of $+35^\circ$, and the constant sp. rotation $[\alpha]_D$ for the equilibrium mixture of anhydrous lactose = $+55.2^\circ$ (water).

Lactose is hydrolyzed by dilute mineral acids to glucose and galactose:



It is also hydrolyzed to the constituent sugars by its specific enzyme *lactase*, found in kephir grains, in some yeasts (*Torulae*), in almonds, etc. Lactose is not hydrolyzed by invertase, maltase or diastase. It is not fermented by brewer's yeast since the latter is unable to hydrolyze it. Certain bacteria, such as lactic acid or butyric acid bacilli, convert lactose probably after hydrolysis with the lactase present into the corresponding acids. Lactose reduces Fehling's solution and with phenylhydrazine gives *lactosazone*, m.p.

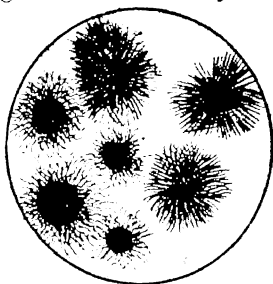


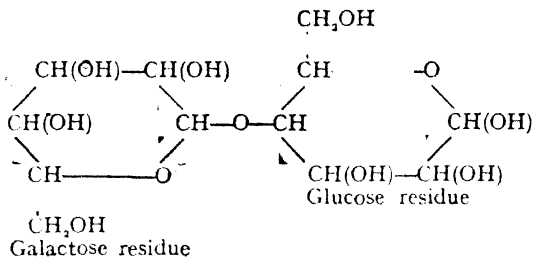
FIG. 35.

200°; the characteristic osazone which *crystallizes out on cooling* (cf. glucose) easily distinguishes this from other sugars (Fig. 35).

Lactose is distinguished from glucose by Barfoed's reagent which is, if at all, very slightly reduced by the former and the reduced Cu_2O forms only a thin film on the surface of the Barfoed's solution. It is also distinguished from glucose or sucrose by *Rubner's test* which is carried out as follows: A solution of the sugar is treated with a solution of lead acetate, boiled for a few seconds and ammonia is added until a white precipitate is formed; on boiling again, the precipitate becomes cream coloured; with sucrose the precipitate remains colourless and with glucose it becomes salmon pink. On oxidation with bromine, lactose gives *lactobionic acid*, the glucose residue being oxidized: $\text{C}_{11}\text{H}_{21}\text{O}_{10}\cdot\text{CHO} \rightarrow \text{C}_{11}\text{H}_{21}\text{O}_{10}\cdot\text{COOH}$. With a stronger oxidizing agent such as nitric acid, lactose gives a mixture of saccharic and mucic acids.

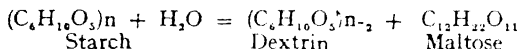
Lactose is used in medicine and sometimes as a diluent of other drugs as also as a food.

According to Haworth and others, lactose has the following structure:



Maltose, Malt Sugar, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$

Occurrence.—This occurs in the free state in small quantities in many plants, and its origin is probably due to hydrolysis of starch by the action of the enzyme *diastase* present in the plant:



Preparation.—Maltose can be easily prepared by the hydrolysis of starch with the help of diastase found in malt extract (see p. 83). A paste of starch (100 g.) with water (400 c.c.) is treated with malt extract (5 g.) and kept at about 60° for one hour. Most of the starch is hydrolyzed to maltose: $2(\text{C}_6\text{H}_{10}\text{O}_5)_n + n\text{H}_2\text{O} = n\text{C}_{12}\text{H}_{22}\text{O}_{11}$. The liquid is heated to boiling, filtered and concentrated in vacuo to a syrup. It is then mixed with 90 per cent alcohol which precipitates the dextrin, the maltose remaining in solution. On removing the alcohol from the filtrate and seeding the residue with some crystals of maltose, the latter crystallizes out. It can be purified further by recrystallization from hot methyl alcohol with the use of animal charcoal.

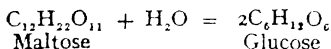


FIG. 36.

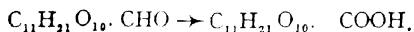
is less sweet than glucose (see p. 211). It shows muta-

Properties and Reactions.—Maltose crystallizes from water in needles with one molecule of water of crystallization. $\text{C}_{12}\text{H}_{22}\text{O}_{11} \cdot \text{H}_2\text{O}$, m.p. 100° . It is readily soluble in water but less so in alcohol and methyl alcohol. It

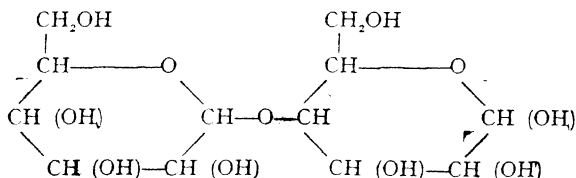
rotation, the constant value being $[\alpha]_D^{20} = +137^\circ$. It reduces Fehling's solution and with phenylhydrazine it forms an osazone with characteristic crystals different from those of glucose or lactose, m.p. 206° (Fig. 36). Maltose is hydrolyzed by dilute mineral acids to two molecules of glucose:



The same hydrolysis takes place with the enzyme maltase. Maltose is fermented by yeast, which first hydrolyzes it to glucose with the help of maltase present in yeast. On oxidation with bromine, maltose gives *maltobionic acid*:

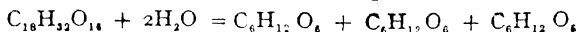


With a stronger oxidizing agent maltose is oxidized to two molecules of saccharic acid. According to Haworth and others maltose has the structure of glucose- α -glucoside:



2. Trisaccharides, $\text{C}_{18}\text{H}_{32}\text{O}_{14}$

e.g., **Raffinose**: occurs in beet root, in cotton seeds, etc., crystallizes in colourless prisms, m.p. $118\text{--}119^\circ$; soluble in water but not in alcohol; has no reducing power; $[\alpha]_D = +104^\circ$ (water); on hydrolysis, it yields fructose, glucose and galactose:



3. Tetrasaccharides, $\text{C}_{24}\text{H}_{42}\text{O}_{21}$

e.g., **Stachyose**: occurs in the roots of some plants, e.g., *Stachys tubrifera* Nd.; forms colourless crystals, m.p. $167\text{--}170^\circ$; sweet in taste and easily soluble in water; has no reducing power; $[\alpha]_D = +148^\circ$ (water); hydrolyzed by mineral acids to glucose, fructose and two molecules of galactose.

Class III. Polysaccharides.

These may be regarded as the condensation products or anhydrides of monosaccharides, which may be either pentoses or hexoses. They possess very high molecular weights and

most of them are amorphous and tasteless. The majority of them are insoluble in cold water and in alcohol. According to the nature of the monosaccharide unit, they are called *pentosans* $(C_5H_8O_4)_n$ or *hexosans* $(C_6H_{10}O_5)_n$. Among the pentosans, which have no medical importance, are compounds like *araban* and *xylan* which occur in plants and give rise to arabinose and xylose respectively on hydrolysis. The hexosans include many compounds such as starch, dextrin, glycogen, inulin, cellulose, etc. The polysaccharides contain free OH groups and yield esters with acetic and nitric acids and methyl ethers with methyl sulphate.

Starch, *Amylum*, $(C_6H_{10}O_5)_n$

Occurrence.—This is widely distributed throughout the vegetable kingdom, the soluble sugar first formed by photosynthesis being ultimately transformed into starch and stored as such. It is thus found in fair amounts in seeds, tubers and rhizomes where it acts as a reserve material for the nutrition of the young plant. *Potatoes* contain 15 to 20 per cent of starch, *rice* 75 to 80 per cent, *wheat* and *maize* 60 to 65 per cent, and so on. Other starches used as food are *arrowroot*, obtained from the rhizomes of *Maranta arundinacea* L., cultivated in the West Indies, *tapioca* obtained from the tubers of cassava (*Manihot utilisima* Pohl.) grown in Southern India, *sago* obtained from the pith of the sago palm (*Sagrus rumphii* Willd.), and *sothi* found in Bengal, prepared from the rhizomes of *Curcuma zeodoria* Rose., etc.

Preparation.—Starch is chiefly prepared by mechanical means from rice, potatoes or maize (or Indian corn), the last forming the largest source from U.S.A. The material is softened, crushed and washed with water through sieves which retain the coarser particles of cellulose, proteins, etc., and allow the starch granules to pass through. The starch granules are allowed to settle, washed with fresh water and finally freed from water by a centrifugal machine and dried at a low temperature.

Properties and Reactions of Starch.—Starch forms a fine white tasteless powder and occurs in plants as minute granules made up of concentric layers around a hilum; the

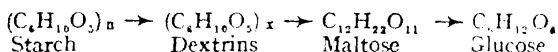
size shape and structure of these granules as seen under the microscope vary with the source of the starch and help in their identification; thus, the granules of wheat or barley starch (diameter 25-35 μ) or potato starch (diam. about 45-100 μ) are much larger than those of rice (diameter 6-10 μ). Starch is insoluble in cold water and in alcohol, and it can be precipitated from its solution in hot water by the addition of alcohol. If a suspension of starch in water is boiled, the granules swell up and burst forming an opalescent solution which becomes pasty on cooling and is called *starch paste*. Starch solution gives a dark blue colour with a solution of iodine in KI; the blue colour, due to an iodine adsorption complex of variable composition, disappears on heating or on adding an alkali and reappears on cooling or acidification. The reaction is very delicate and is used both for the detection of starch and also for very small quantities of iodine. Starch solution does not reduce Fehling's solution. It is dextrorotatory; sp. rotation = $+200^\circ$ (dissolved in HCl).

Starch granules are composed of (1) *amylose* (α -amylose) which occurs in the interior of the granules and forms about 66 per cent of the starch; it is soluble in cold water and gives a clear bright blue colour with iodine; it is hydrolyzed by malt extract completely into maltose; (2) *amylopectin* (β -amylose) which occurs in the envelope of the granules and forms about 33 per cent of the starch; it is insoluble in cold water and swells up in hot water giving the mucilaginous character to starch paste; it gives a violet colour with iodine solution; amylopectin contains very small quantities of a phosphoric acid complex (e.g., 0.084 per cent of P in potato starch).

Soluble Starch.—When starch granules are treated with dilute HCl (about 10 per cent) for several hours, they become soluble in hot water without forming a paste. This property is utilized in making soluble starch. Pure starch (100 g.) is treated with dilute HCl (200 c.c., about 7.5 per cent) at room temperature for 6 or 7 days, stirring the mixture regularly; the acid is removed by decantation, washed free from HCl, and dried.

Hydrolysis of Starch.—Saliva, which contains an enzyme *amylase* of *diastase* (formerly known as *ptyalin*), acts upon

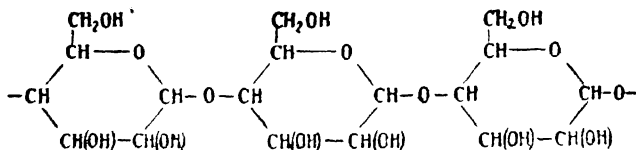
starch and hydrolyzes it in stages, first to dextrin and then to maltose. The amylase present in pancreatic juice (formerly known as *amylpsin*) hydrolyzes starch in the same way to maltose. Amylase (diastase) is unable to hydrolyze starch beyond the maltose stage. *Taka diastase* (prepared from some species of the fungus, *Aspergillus*), which contains both diastase and maltase, can however convert starch into glucose, the hydrolysis of maltose to glucose being done by maltase. On heating with dilute mineral acids, starch is hydrolyzed as follows, the final product being glucose:



The content of starch is estimated by the amount of glucose formed by its hydrolysis.

Uses of Starch.—Apart from its use as food (e.g., rice, wheat, potato, etc.), starch is the starting material for the preparation of glucose, corn syrup, dextrin, ethyl alcohol, etc. It is also used in sizing paper and in finishing cotton fabrics.

Structure of Starch.—According to Haworth and others, the starch molecule is composed of a regular chain of α -glucopyranose units and the length of the starch molecule is stated to contain at least 24 to 30 glucose units:



Dextrin, British Gum, $(\text{C}_6\text{H}_{10}\text{O}_5)_x$

Dextrins are polysaccharides produced by the partial hydrolysis of starch, either by dilute mineral acids, by heat or by enzymes. Thus when pure starch is heated to about 200° , it is converted into dextrin which does not give the blue colour with iodine; the same change takes place when bread is toasted or linen is ironed. The dextrins are intermediate in complexity between starch and maltose, and are composed of several glucose units.

Dextrin is generally prepared on a large scale by moistening starch with dilute nitric acid, stirring the paste and heating for some time at about 120° . It is an amorphous, tasteless power, soluble in water but insoluble in alcohol. It is dextrorotatory and hence the name. It is also known as *starch gum* and *British gum* (to distinguish it from natural gums such as gum acacia, gum arabic, etc.) and is used in the preparation of adhesives. It is probable that a number of dextrans are formed during the gradual hydrolysis of starch, and two of these are well known: (1) *erythro-dextrin*, which gives a reddish brown colour with iodine and (2) *achroo-dextrin*, which gives no colour with iodine. On hydrolysis with enzymes or with dilute mineral acids, the dextrans yield maltose and ultimately glucose.

Glycogen, Animal Starch, $(C_6H_{10}O_5)_n$

Occurrence.—This polysaccharide is found as a reserve food material in fair amounts in the liver and in small quantities in the muscles of all animals. Oysters and other molluscs are known to be rich in glycogen. Although known as animal starch, it is found in plants such as yeasts and certain fungi. After a meal rich in carbohydrates, the glycogen content of the liver is increased due to the absorbed glucose being condensed into and stored as glycogen; the liver can convert the glycogen again into glucose when necessary. During muscular work, the amount of glycogen in muscle is diminished on account of its partial conversion into lactic acid (*vide* lactic acid).

Preparation.—Livers from animals fed on an excess of carbohydrate diet are cut into fine pieces, washed with normal saline, and poured into boiling water containing a little acetic acid in order to coagulate the proteins and stop the action of the hydrolyzing enzymes. The pieces are then finely ground up and extracted with boiling water, the aqueous extract treated with trichloroacetic acid in order to precipitate all the proteins, and filtered. The glycogen is then precipitated from the filtrate with the addition of an equal volume of alcohol.

Properties and Reactions.—Glycogen is a tasteless, amorphous, white power. It dissolves in hot water giving an opalescent solution which is dextrorotatory; $[\alpha]_D^{25} = +196^{\circ}$. With iodine solution it gives a reddish brown or mahogany

brown colour. It does not reduce Fehling's solution and is not fermented by yeast. It is composed of several glucose units and as in the case of starch, it is hydrolyzed by appropriate enzymes or by dilute mineral acids gradually into dextrin, maltose and glucose. Glycogen is stated to have the same structure as starch but it has a lower molecular weight and is stated to have a chain consisting of a minimum of 12 glucose units.

Inulin, $(C_6H_{10}O_5)_n$

Occurrence.—Inulin occurs in the tubers of the dahlia (*Dahlia variabilis* Desf.), Jerusalem artichoke (*Helianthus tuberosus* L.) and chicory (*Cichorium intybus* L.), and has more recently been isolated by Ghosh from the roots of *ket* (*Saussurea lappa* Clarke) growing in Kashmir with an yield of nearly 18 per cent. Inulin forms the main source of fructose.

Preparation.—Dahlia tubers are minced and extracted with boiling water in the presence of some calcium carbonate and filtered hot through muslin. The filtrate is treated with a solution of lead acetate to remove some impurities, filtered and treated with H_2S to remove the excess of lead. The PbS is filtered off and the filtrate concentrated to a small bulk when the inulin is precipitated specially with the addition of some alcohol. It can be purified further by redissolving in hot water, decolorizing with animal charcoal, filtering, concentrating and precipitating with alcohol.

Properties and Reactions.—It is a white, tasteless, crystalline power. It is soluble in hot water, the solution being lævorotatory, $[\alpha]_D = -35^\circ$. It consists of fructose units, and is hydrolyzed by dilute mineral acids or by the specific enzyme *inulase* to d-fructose. The chain length of inulin is stated to consist of about 30 fructofuranose units.

Araban, $(C_6H_8O_4)_n$: This is also a polysaccharide and a *pentosan* found in many plant gums, such as cherry gum (from *Prunus cerasus* L.), gum arabic (from *Acacia senegal* Willd.), gum tragacanth (from *Astragalus gummifer* Labill.), etc. It is a white amorphous mass, soluble in water, and is hydrolyzed by mineral acids to d-arabinose.

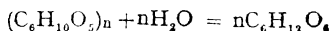
Xylan, Wood Gum, $(C_5H_8O_4)_n$: This is also a polysaccharide and a *pentosan* found in straw, grasses, wood of various trees, in bark, root, bran of seed, etc., and is stated to be, next to cellulose, the most abundant of plant constituents. It is a white amorphous powder almost insoluble in water. It is hydrolyzed by mineral acids to 1-xylose.

Cellulose, $(C_6H_{10}O_5)_n$

Cellulose is a polysaccharide and a *hexosan*. It forms a large portion of the cell walls of plants. Cotton wool contains about 99 per cent of pure cellulose and good filter paper is almost pure cellulose.

Pure cellulose is a white hygroscopic substance which is insoluble in water and in alcohol. It does not give any colour with iodine solution. It gives the Thymol Test (see p. 195). It is soluble in (a) a solution of zinc chloride in conc. HCl, and (b) in an ammoniacal solution of cupric oxide, known as *Schweitzer's reagent*, in which the fibres first swell up and then dissolve.

Cellulose is not easily hydrolyzed by ordinary enzymes; it is, however, attacked by certain bacteria, usually found in the intestines of herbivora, with the formation of methane, hydrogen, carbon dioxide, etc. When heated with dilute mineral acids (say, 5 per cent H_2SO_4) under pressure, cellulose is hydrolyzed to glucose; this method is used on a large scale for the manufacture of alcohol:



Besides its use in cotton industry, cellulose is largely utilized in manufacturing paper. When treated with a mixture of concentrated nitric and sulphuric acid (3:1), nitric acid esters or *nitro-celluloses* are produced. The higher nitro-celluloses are used in making *explosives* such as gun-cotton $C_6H_7O_2(O.NO_2)_3$ which is the trinitrate used in making 'blasting gelatine', cordite, etc. The lower nitric acid esters or *pyroxylin*s $[C_6H_7O_2.OH(O.NO_2)_2]$ dissolved in a mixture of alcohol (one volume) and ether (three volumes) form the *collodion* used in medicine as 'surgical collodion' for sealing the puncture after an injection. The lower nitro-celluloses mixed with camphor and a few other substances yield *celluloid*, used in various industries. Cellulose is also largely used in making *artificial silk* by dissolving it in a suitable solvent, e.g., Schweitzer's reagent, squirting the solution through fine holes and then evaporating the solvent.

A strong solution (15–20%) of NaOH produces a curious thickening and gelatinization of the fibre which

becomes translucent and the process is known as '*mercerization*' (after John Mercer). The mercerized fabric is insoluble in 10% NaOH but natural silk is soluble in the same.

The acetic acid ester or *cellulose acetate* is used in making artificial silk (technically called '*acetate silk*' or '*celanese*'), cinema films, and *dopes*, a kind of varnish used in aeroplane wings. The transparent sheets or foils of cellulose acetate are used in preserving old books and documents by *lamination*.

The hard '*fibre*' used in making trunks, suit cases, etc., is prepared by compressing sheets of paper which have been gelatinized by ZnCl_2 and other reagents.

The *parchment paper* is made by gelatinization of paper with H_2SO_4 . The transparent paper of commerce (different from cellulose acetate foil) is made by a similar process.

As regards the *structure* of cellulose, it is stated to consist of a regular chain of β -glucose units joined in a glucosidic union, and the cellulose molecule consists of about 200 glucose units in the chain.

Class IV. Complex Polysaccharides.

1. Hemicelluloses.

These are found along with cellulose in plants. They are insoluble in water and are hydrolyzed by dilute mineral acids, more readily than cellulose, into sugars other than glucose, generally galactose, mannose, arabinose or xylose; e.g., galactan, mannan, agar-agar, etc.

Agar-agar or *Japanese Isinglass* or '*Chinese root*' is a galactan prepared from certain sea-weeds (Rhodophyceae). Agar-agar is odourless and tasteless and insoluble in cold water. It is soluble in hot water, and on cooling the solution sets to a thick jelly. It is largely used to prepare solid media for culture in bacteriological work. It is also used to thicken milk, cream, ice-cream, etc. On hydrolysis of agar-agar, the main product is galactose, and it is stated to be the sulphuric acid ester of a linear polygalactan. A sea weed *Gracillaria confervoides* Greville (Fam. Rhodophyceae), found in the Chilka Salt Lake, has been shown to yield agar-agar conforming to the B.P. standard (Bal, Basu & Chakravarty).

Jute, the fibre obtained from the plants *Corchorus capsularis* L., and *Corchorus olitorius* L., is stated by Sircar to possess the following approximate percentage composition: α -cellulose 60.0, hemicellulose

20.0, lignin 11.0, polyuronides 5.0, nitrogenous matter 1.3, fats and waxes 1.0, ash 1.2, miscellaneous 0.5.

2. Gums. These are exudations of certain plants and are transparent, amorphous substances which dissolve in water forming a slimy solution. They are insoluble in alcohol and are, therefore, precipitated from an aqueous solution on adding alcohol. They are composed of hexoses and pentoses together with uronic acids (see p. 213), and are mostly combined with K, Ca or Mg as their salts. Some of the gums, such as *gum arabic* and *gum tragacanth*, are used in medicine for emulsifying oils. On hydrolysis, both gum arabic and gum tragacanth yield galactose, arabinose and uronic acids.

3. Mucilages. These are closely related to the gums and the name is applied to those substances which dissolve in water to form a slimy liquid.

4. Pectin. Pectin is a water-soluble gelatinizing colloid. It is stated to be the methyl isopropenyl ester of pectic acid and is found in fruits, such as guava, apple, black-berry, goose-berry, currants, oranges, etc., and is the cause of the gelatinization often seen after boiling these fruits. This property of jellification, specially, in the presence of acids such as citric acid and cane sugar, is used in making fruit jellies and jams. The pectins are hydrolyzed by mineral acids first to *pectic acid* which on further hydrolysis gives galactose, arabinose and uronic acids, generally galacturonic acid.

CHAPTER XVIII

GLYCOSIDES, SAPONINS AND BITTER PRINCIPLES

Glycosides.

General Considerations.—The term *glycoside* is applied to a large number of substances, both naturally occurring and synthetic, which split up on hydrolysis into a reducing *sugar* and a *non-sugar component* called *aglucone* or *aglycone*. The term *glucoside* now denotes a specific name applied only to those glycosides in which the sugar component is glucose, the other term, *glycoside*, being a general name for the group irrespective of the sugar present.

The *sugar component* in a glycoside may be a disaccharide, a hexose, a pentose or even a sugar of special constitution as found in the *Digitalis* glycosides. The *aglucone* or the non-sugar component varies a good deal in nature and the *classification* of the glycosides is based upon the nature of the aglucone component. These aglucones may be phenols, alcohols or aldehydes of the aromatic series, various colouring matters (such as, hydroxyanthraquinones, coumarins, flavones, flavonols, flavonones, xanthones, anthocyanins, etc.), compounds like digitoxigenin, digitaligenin, strophanthidin, etc., of complex composition, isothiocyanates (or mustard oils), KHSO_4 , hydrocyanic acid, etc. The glycosides which yield HCN on hydrolysis are given the special name of *cyanogenetic* (or cyanophoric) glycosides.

The glycosides are found widely distributed in plants, chiefly in the fruit, bark and roots. They are generally accompanied by their specific enzymes, found in separate cells, which hydrolyze them.

Préparation.—The plant material is extracted with hot water or hot alcohol in order to prevent the action of the enzymes, and some powdered calcium carbonate is added to

prevent hydrolysis by the free acids present. The extract is concentrated to a small bulk in vacuo, when the glycoside usually crystallizes out. In some cases, the aqueous extract is treated with a solution of lead acetate or basic lead acetate or preferably with freshly precipitated lead hydroxide in order to remove the colouring matter, the excess of lead removed by H_2S , the filtrate from PbS neutralized and then concentrated in vacuo to a syrup. The glycoside may then be extracted with some organic solvent from which the pure substance crystallizes out. In practice, however, no general method can be laid down as the process of isolation and purification varies a good deal with the properties of the glycoside.

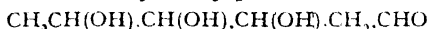
Properties and Uses.—The glycosides are usually colourless, crystalline solids, soluble in water and alcohol but insoluble in ether. Most of them are laevorotatory and have a slightly bitter taste. They are hydrolyzed by dilute mineral acids or by specific enzymes to a reducing sugar and a non-sugar component (aglucone). Some of these glycosides are active poisons, such as those found in *Digitalis*, *Strophanthus*, *Oleanders*, *Antiaris*, etc. Others act as poisons owing to the liberation of HCN , others again are harmless. Some of the glycosides, such as digitoxin, strophanthin, ouabain, salicin, etc., are used in medicine.

Digitalis Glycosides.

These are found in the leaves and seeds of the foxglove, *Digitalis purpurea* L., now cultivated in India (Kashmere, Nilgiris, Mungpoo). The *tincture of digitalis*, a standardized alcoholic extract of the leaves, is used in medicine to decrease the frequency and increase the force of the heart beat. The leaves contain several *glycosides*, such as digitoxin, gitoxin, digitalin, gitalin, etc., and some *sapomins* such as digitonin, gitonin, etc. There are no accurate chemical methods for testing the activity of these glycosides and they are usually assayed by biological methods.

Digitoxin $\text{C}_{41}\text{H}_{64}\text{O}_{13}$ this glycoside is the most active principle of the leaves; on hydrolysis it yields 3 molecules of the sugar *digitoxose*, $\text{C}_6\text{H}_{12}\text{O}_4$, and one molecule of the

aglycone *digitoxigenin* $C_{23}H_{34}O_4$. Digitoxose has been shown to be a deoxymethylpentose of the structure:



N.B.—The leaves of *Digitalis lanata* Ehrh, which contain many of the glycosides found in *Digitalis purpurea* and have been recently introduced into India, are stated to possess a physiological activity 2 to 3 times that of the international standard leaf. This is stated to contain the active glycoside *lanadigin*.

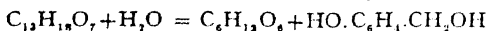
Strophanthus Glycosides.

The seeds of various species of *Strophanthus*, growing in tropical eastern Africa and used as arrow poison, contain glycosides which have a toxic action on the heart. One such glycoside *g-strophanthin* or *ouabain*, isolated from the seeds of *Strophanthus gratus* Franch., is used in medicine in heart troubles as well as a standard for biological assays. *Ouabain* $C_{30}H_{46}O_{12}$ is hydrolyzed by acids to rhamnose $C_6H_{12}O_5$ and the aglycone $C_{24}H_{36}O_8$.

Salicin, $C_{13}H_{18}O_7$

This glucoside occurs in the bark of the willow tree (*Salix alba* L., *Salix fragilis* L., etc.) as also in the bark of the poplar (*Populus alba* L., etc.) and in other plants. It can be prepared by extracting the bark with boiling water, concentrating the extract to a small bulk, digesting with litharge, filtering and concentrating the filtrate to a syrup. On cooling, salicin separates out, and it is purified by recrystallization from water.

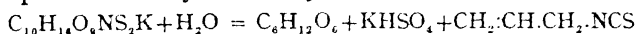
Salicin crystallizes in colourless prisms and has a bitter taste, m.p. 201° ; $[\alpha]_D^{20} = -62.5^\circ$ (in 5 per cent aqueous solution). It is sparingly soluble in cold water but more easily soluble in hot water or alcohol. It is used in the treatment of rheumatism and also as an antipyretic. On hydrolysis, by emulsin or by dilute mineral acids, it gives glucose and saligenin (o-hydroxybenzyl alcohol):



Sinigrin, $C_{10}H_{16}O_9NS_2K$.

This glucoside is found in the seeds of the black mustard, *Brassica nigra* Koch. It crystallizes from alcohol

in colourless needles, m.p. 129° ; $[\alpha]_D^{20} = -15.7^{\circ}$ (water). It tastes bitter and is easily soluble in water. On hydrolysis with *myrosin*, the enzyme present in black mustard, or with dilute mineral acids, it gives glucose, potassium hydrogen sulphate and allyl isothiocyanate:

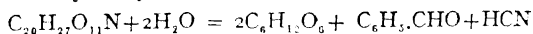


Amygdalin, $C_{20}H_{27}O_{11}N$

This glucoside occurs, along with its specific enzyme *emulsin*, in bitter almonds (*Prunus amygdalus* var. *amara* Sock.) as well as in the kernels of apricot (*Prunus armeniaca* L.), peach (*Prunus persica* Sieb. et Zucc.), English plum (*Prunus domestica* L.), etc. It belongs to the group of glucosides which give hydrocyanic acid as one of the products of hydrolysis. These cyanogenetic glucosides may be harmless when intact but may cause poisoning due to the liberation of HCN on hydrolysis in the stomach of men and animals.

Amygdalin can be easily prepared from bitter almonds. The crushed kernels are freed from fatty oil by extraction with petroleum ether and then extracted with boiling 95 per cent alcohol; the alcoholic extract is concentrated to a small bulk and treated with about half the bulk of ether; the precipitate of amygdalin thus obtained is purified by recrystallization from alcohol.

Amygdalin is a colourless crystalline bitter substance soluble in water and in hot alcohol, m.p. 215° ; $[\alpha]_D = -41.5^{\circ}$ (water). On hydrolysis, it yields glucose, benzaldehyde and hydrocyanic acid:



Other common cyanogenetic glucosides are,

Linamarin found in the linseed, mostly in the immature seeds; split up on hydrolysis by the enzyme *linase* into HCN, acetone, and glucose. **Dhurrin** is found in the young *Sorghum vulgare* Pers. (Ind.—*Jowar*, *Gama*) plant, the common green fodder for cattle in this country. It has been recently shown by Bagchi to be present in *bamboo shoots*, and it splits up into HCN, p-hydroxybenzaldehyde and glucose. Both the glucosides, linamarin and dhurrin, cause fatal HCN poisoning in cattle in Bengal and other provinces. The specific enzyme for dhurrin is stated to be *emulsin*. The amount of HCN in bamboo shoots may be as high as 0.8 per cent.

The Saponins.

These form a group of glycosides fairly widely distributed in the vegetable kingdom. On hydrolysis, they yield a *sugar* and a non-sugar component known as *sapogenin*.

They are usually white or cream coloured powders, soluble in water and in hot alcohol but insoluble in ether, chloroform or benzene. They possess a bitter taste and the dry powders irritate the nose. They reduce the surface tension of water and their aqueous solutions produce a persistent froth on shaking (hence the name saponin), and form emulsions with oils or resins and thus prevent the deposition of finely divided particles. They are toxic to cold-blooded animals like frogs and fishes and hæmolyze red blood corpuscles, the hæmolytic action being utilized in the estimation of saponins. They form additive compounds with cholesterol. The more poisonous saponins are known as *sapotoxins*. The saponins are divided into two classes (1) *neutral saponins*, which are precipitated by basic lead acetate, and (2) *acid saponins* which are precipitated by neutral lead acetate.

Some of the drugs used in medicine, such as sarsaparilla, squill, quillaia, senega, etc., contain saponins as their active components. *Ritha* or Soap-nut (*Sapindus mukorossi* Gaertn. or *S. trifoliatu*s L.) is used in washing as a substitute for soap and its action is due to the emulsifying power of the saponins contained in the nut.

Bitter Principles.

They form a group of naturally occurring substances with a bitter taste, which are neither glycosides nor alkaloids. They are used in medicine either as anthelmintics or as bitter tonics. The best known compound is *santonin* $C_{15}H_{18}O_3$, a bitter substance found in the flowering tops of *Artemisia maritima* L., growing in Kashmere, Russian Turkistan, etc., and is used in the treatment of round worms; this is a naphthalene derivative. Other bitter substances used as drugs are found to occur in Quassia, Chirata, Gentian, Aloes, Andrographis (*Kalmegh*), Colocynth, etc.

CHAPTER XIX

CYANOGEN COMPOUNDS

The cyanogen compounds include a group of substances derived from the monovalent radical $-C \equiv N$. This radical is very similar in its behaviour to the monovalent halogen atoms; thus,



Cyanogen, C_2N_2 , $\text{N} \equiv \text{C}-\text{C} \equiv \text{N}$

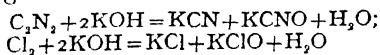
Occurrence and Preparation.—This is normally present in the gases from blast furnaces and in crude coal gas. It is formed when ammonium oxalate is dehydrated by heating with phosphorus pentoxide:



It can be prepared in the laboratory by heating dry mercuric cyanide to a dull red heat:



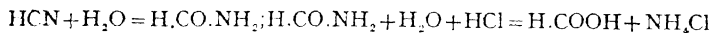
Properties and Reactions.—It is a colourless gas with a peculiar smell resembling that of bitter almonds. The gas burns with a bluish-red flame, forming carbon dioxide and nitrogen. It is intensely poisonous. It is soluble in alcohol and water. The watery solution, however, does not keep but decomposes with the formation of ammonium oxalate and a complex acid, *azulmic acid*, which comes down as a brown precipitate. On passing cyanogen into potassium hydroxide solution, potassium cyanide and cyanate are formed, the reaction being similar to that with chlorine:



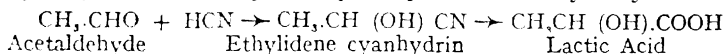
for an adult being only 0.06 gram of the acid (or 0.15 gram of KCN). The soluble salts are also extremely poisonous.

Hydrocyanic acid is used as an insecticide for fumigating ships, carriages, ware-houses, etc., usually mixed with cyanogen chloride to render it less dangerous as the cyanogen chloride makes its presence felt by its irritating properties and so warns the workers of the presence of this poisonous gas. Hydrocyanic acid is used in medicine (2 to 5 drops) in a very dilute solution, the B.P. *acidum hydrocyanicum dilutum* containing about 2 per cent (w/w) of HCN. Another preparation used in medicine is cherry laurel water or *aqua laurocerasi*, which contains about 0.1 per cent of HCN.

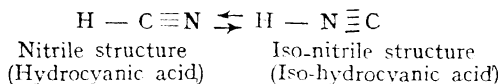
On hydrolysis with hydrochloric acid, first formamide and then formic acid and ammonium chloride are formed; hydrocyanic acid is therefore the nitrile of formic acid or formonitrile:



With most aldehydes and ketones, it combines direct forming cyanhydrins which yield hydroxy acids on hydrolysis.



As regards the structure of HCN, the pure anhydrous acid is stated to be an equilibrium mixture of two *tautomeric forms*, and at ordinary temperatures the nitrile structure is known to predominate.



The structure of isohydrocyanic form is also shown as $\text{H} - \text{N} \equiv \text{C}$ (vide electronic formulae in p. 42).

Tests for HCN and Cyanides.—

(1) The solution is made slightly alkaline with 2 or 3 drops of 5% caustic soda and treated with a few drops of freshly prepared 5% solution of ferrous sulphate and 1 or 2 drops of 3% ferric chloride solution. The mixture is then allowed to stand for 2 minutes, gently heated and acidified with HCl. Sufficient HCl only is used to clear the liquid—there should not be a great excess of acid. There is either a greenish-blue colour or a precipitate of Prussian blue (Prussian blue reaction). Traces of cyanide may show only after 15-30 mts.,

but on standing overnight a blue precipitate settles out. Too much HCl retards the formation of Prussian blue which dissolves in conc. HCl giving a yellow colour. (See Toxicology, Chapt. 34).

(2) The solution is heated with 10-15 drops of yellow ammonium sulphide for 15 minutes on the water bath. Acidify with dilute HNO_3 . Filter through double filter paper till the filtrate is clear. To the filtrate ferric alum solution is then added drop by drop until the blood-red colour of ferric sulphocyanide is obtained.

Both these tests are very delicate and can be used for testing minute quantities of HCN obtained by acidification and distillation from foodstuffs or viscera or any substance suspected to contain cyanides. For further tests, see pp. 462-465.

Cyanides.—The cyanides, *i.e.*, salts of HCN, of the alkali metals and alkali earth metals are soluble in water; their aqueous solutions are alkaline in reaction owing to hydrolysis. The cyanides of the heavy metals, with the exception of mercuric cyanide, are insoluble in water. The alkali cyanides are all strong poisons.

Potassium Cyanide, KCN

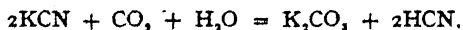
Preparation.—This can be prepared in the laboratory by strongly heating potassium ferrocyanide in the absence of air. A better yield is obtained by fusing potassium ferrocyanide with potassium carbonate:



The KCN can be isolated by extracting the residue with hot dilute alcohol and recrystallizing.

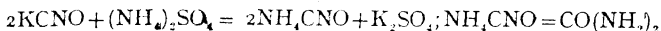
Properties and Uses.—Potassium cyanide crystallizes from water in colourless plates. It is readily soluble in water and deliquesces in air but is very little soluble in absolute alcohol. It is strongly alkaline in reaction due to hydrolytic dissociation, and is extremely *poisonous*. It melts at a dull red heat and volatilizes without decomposition at a high temperature. It is used in electroplating and in the extraction of gold. In organic chemistry the chief use of potassium cyanide is in the preparation of nitriles and other cyanogen compounds.

KCN, in the presence of moisture, is decomposed by CO_2 of air with the formation of K_2CO_3 and liberation of HCN:



The free HCN liberated in the above reaction also hydrolyzes slowly to ammonium formate in the presence of moisture.

Potassium Cyanate, KCNO.—This is prepared by melting KCN with litharge PbO. The mass is extracted with water and recrystallized from dilute alcohol. It is a stable salt and crystallizes in colourless plates readily soluble in water and is insoluble in absolute alcohol. The *ammonium cyanate*, which is formed by the double decomposition of potassium cyanate and ammonium sulphate, is readily transformed into urea (see urea):

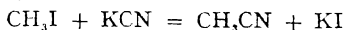


Mercury Fulminate, $\text{Hg}(\text{C} \equiv \text{NO})_2$, a compound obtained by adding alcohol to a solution of mercury in strong nitric acid. It explodes on percussion and is therefore used in making percussion caps and as a detonator. It is an isomer of *mercury cyanate*.

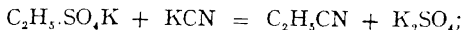
Double Cyanides.—When a solution of KCN is gradually added to a solution of AgNO_3 , there is at first a precipitate of AgCN which dissolves with further addition of KCN forming a double cyanide AgCN.KCN . Similar double cyanides are formed with gold and some other metals.

Alkyl Cyanides, Nitriles, $\text{R.C} \equiv \text{N}$

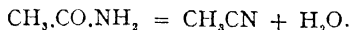
These can be prepared (1) by heating an alkyl iodide with potassium cyanide in alcoholic solution:



(2) by heating an alkyl sulphate with KCN:



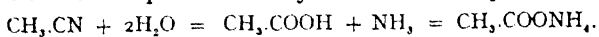
or (3) by distilling an acid amide with a dehydrating agent like phosphorus pentoxide:



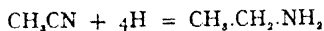
The nitriles are usually colourless liquids or solids with an ethereal smell. When hydrolyzed they take up two molecules of water and yield ammonium salt of the corresponding acid.

As CH_3CN is hydrolyzed to acetic acid it is called acetonitrile. HCN is hydrolyzed to formic acid and HCN is therefore known as formonitrile. Similarly, NC.CN is oxalonitrile, $\text{CH}_3\text{CH}(\text{OH}).\text{CN}$ is lactonitrile, and so on.

Thus the hydrolysis of nitrile to carboxylic acid is of considerable importance in synthetic chemistry.



By reduction with sodium and alcohol the nitriles yield primary amines:

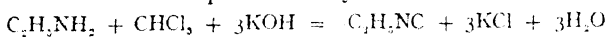


Alkyl-isocyanides, Isonitriles. Carbylamines, $\text{R} - \text{N} \equiv \text{C}$

These are isomeric with the nitriles, but the alkyl group is attached to nitrogen. They may be prepared (1) by heating an alkyl iodide with silver cyanide:

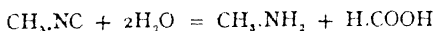


(2) by heating chloroform with a mixture of a primary amine and alcoholic potassium hydroxide,

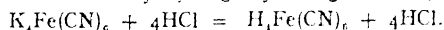


The second reaction clearly elucidates the structure of the isonitriles

The isonitriles are colourless liquids with a very disagreeable odour. On hydrolysis they yield formic acid and primary amines:

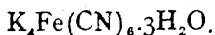


Complex Cyanides.—These are really the metallic salts of complex acids, hydroferrocyanic acid $\text{H}_4\text{Fe}(\text{CN})_6$ and hydroferricyanic acid $\text{H}_3\text{Fe}(\text{CN})_6$. The most important are the potassium salts described below. These compounds are not poisonous and on treatment with acids in the cold they do not evolve hydrocyanic acid; the alkali metal is substituted by hydrogen yielding the complex acid:



The hydroferrocyanic acid and hydroferricyanic acid are strong acids but they are not found in the free state. They contain the acid radicle or negative ion $\text{Fe}(\text{CN})_6$. Their salts are stable. The Zn, Cu and Ferric salts of hydroferrocyanic acid are insoluble in water while K and Na salts are soluble.

Potassium Ferrocyanide, Yellow Prussiate of Potash,

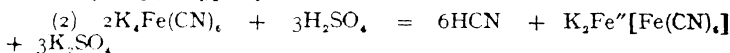
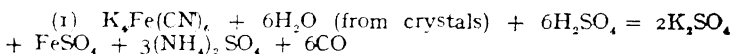


This can be prepared by fusing together some scrap iron, nitrogenous animal refuse (horn shavings, hoofs, dried blood, hair, etc.), and crude potassium carbonate. The fused mass is extracted with water, filtered and concen-

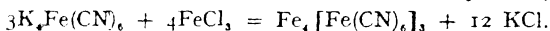
trated, when the salt crystallizes as large lemon-yellow prisms with 3 molecules of water.

Most of the potassium ferrocyanide on the market is now prepared as a bye-product from the gas works and coke ovens. The hydrocyanic acid present is taken up in the iron oxide purifiers with the formation of ferric ferrocyanide; this is treated with hot milk of lime forming a soluble calcium ferrocyanide which is then converted into potassium ferrocyanide by potassium carbonate.

Potassium ferrocyanide is readily soluble in water and is not poisonous. If chlorine gas is passed through a solution of potassium ferrocyanide it is oxidized to potassium ferricyanide. It is used in the preparation of cyanogen compounds; in calico printing, and as a reagent. When warmed with conc. H_2SO_4 it gives CO but when boiled with dilute H_2SO_4 , HCN is formed:

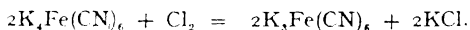


FeCl_3 gives a precipitate of ferric ferrocyanide,

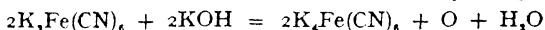


Potassium Ferricyanide, Red Prussiate of Potash,
 $\text{K}_3\text{Fe}(\text{CN})_6$

This is prepared by passing chlorine gas into a solution of potassium ferrocyanide. The salt is purified by crystallization from water:



Potassium ferricyanide is a dark-red crystalline substance, soluble in water. In alkaline solution it acts as a mild oxidizing substance, potassium ferrocyanide being reformed. It is used as a common laboratory reagent



Sodium Nitroprusside, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$

This is prepared by treating potassium ferrocyanide with strong nitric acid and then adding sodium carbonate to neutralize it. It crystallizes in ruby-red prisms soluble in water and is a useful reagent in organic analysis. With an alkaline solution of a sulphide, a freshly

prepared solution of sodium nitroprusside gives a purple colour—this is a very delicate test for sulphides.

Cyanic Acid HCNO

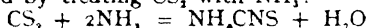
Cyanic acid is a tautomeric substance and is represented by either of the two formulas, $\text{HO}-\text{C}\equiv\text{N}$ or $\text{H}-\text{N}=\text{C}=\text{O}$ and the derivatives are, therefore, known as cyanates and isocyanates. It is a colourless liquid unstable above 0°C . At temperatures above 0° , the liquid polymerizes to *cyanuric acid* (CNOH), which on heating is depolymerized to cyanic acid and then decomposes into NH_3 and CO_2 . *Fulminic acid* $\text{C} \equiv \text{N.OH}$ is an isomer of cyanic acid.

Potassium Thiocyanate, Potassium Sulphocyanate, Potassium Sulphocyanide, KCNS.

Traces of soluble thiocyanates occur normally in saliva and in milk and is also excreted in urine. Potassium thiocyanate is prepared by fusing potassium ferrocyanide with sulphur and potassium carbonate. The mass is extracted with hot alcohol and filtered; on cooling, potassium thiocyanate separates as colourless crystals easily soluble in water. It gives a blood-red colour with ferric salts in faintly acid solutions, forming ferric thio- or sulphocyanate. This is a very delicate test for ferric salts.

Ammonium Thiocyanate, NH_4CNS

It is prepared by treating CS_2 with NH_3 :



Mercuric thiocyanate, $\text{Hg}(\text{CNS})_2$. It is obtained by adding HgCl_2 to a solution of KCNS , as a greyish amorphous precipitate. It is moulded into pellets and then dried which when ignited form voluminous, curled up snake-like tubes of ash popularly known as "Pharaoh's serpents."

Alkyl Thiocyanates, $\text{R}-\text{S}-\text{C}\equiv\text{N}$

Ethyl Thiocyanate, $\text{C}_2\text{H}_5\text{SCN}$. Prepared by distilling potassium thiocyanate with ethyl sulphate. This is a colourless liquid with a leek-like odour, b.p. 142° ; almost insoluble in water.

Alkyl Isothiocyanates, Mustard Oils, R.N:C:S

Allyl Isothiocyanate, Mustard Oil, $\text{CH}_2:\text{CH}.\text{CH}_2.\text{N:C:S}$. This is an allyl ester of iso-thiocyanic acid, found in black mustard seeds (*Rai*) and also in rape seeds as a glucoside known as sinigrin from which it can be obtained by hydrolysis (see sinigrin). It can be synthesized by the action of allyl iodide upon potassium thiocyanate; the allyl thiocyanate first formed is changed into the isothiocyanate on distillation. It is a liquid with a pungent smell and lachrymatory property, b.p. 151° , and when applied to the skin forms painful blisters. The characteristic smell of ordinary edible mustard oil (fixed oil used in Bengal for cooking purposes) is due to the presence of allyl isothiocyanate.

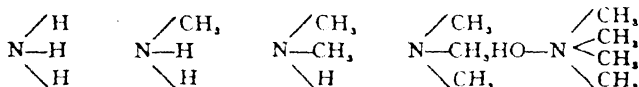
Cyanamide, $N \equiv C.NH_2$.

Its calcium derivative, calcium cyanamide $CaCN_2$, or $Ca:N.C:N$ is important as an artificial fertilizer. In the soil, calcium cyanamide in the presence of CO_2 is converted by bacterial action into ammonium carbonate through the intermediate stage of urea. It is manufactured by passing nitrogen through calcium carbide at a very high temperature: $CaC_2 + N_2 = CaCN_2 + C$. When treated with steam, it is decomposed into ammonia and calcium carbonate and is thus a source of synthetic ammonia. The fact that calcium cyanamide forms urea [$Ca:N.C:N + CO_2 + H_2O = CO(NH_2)_2 + CaCO_3$] is interesting. When treated with ammonia it forms guanidine (see), a decomposition product of proteins.

CHAPTER XX

THE AMINES

These compounds may be considered to be derived from ammonia by the substitution of one or more hydrogen atoms by alkyl groups. According as to whether one, two or three hydrogen atoms are substituted by alkyl groups, primary-, secondary- or tertiary- amines are obtained. Thus a *primary amine* possesses the monovalent *amino group* —NH_2 , attached to one carbon atom, a *secondary amine* possesses the divalent *imino group* $=\text{NH}$, attached to two different carbon atoms, and a *tertiary amine* would be one in which all the hydrogen atoms of ammonia are replaced by alkyl groups ($\equiv\text{N}$). The four hydrogen atoms of ammonium hydroxide may also be substituted by alkyl groups giving a *tetra alkyl ammonium hydroxide* or quaternary ammonium hydroxide, e.g.,



The H atoms of NH_3 may be replaced either by the same alkyl groups giving *simple amines*, or by *different alkyl groups* giving *mixed amines*.

Occurrence of Amines.—The simpler amines have been prepared synthetically, but many have been found in the animal and vegetable kingdom. Some of them possess strong physiological action and are of great biochemical importance.

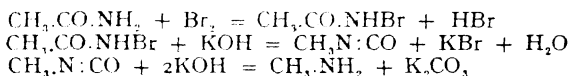
Synthetic Methods of Preparing Amines.

(1) When an alkyl iodide is heated with alcoholic ammonia in a sealed tube, the H atoms of ammonia are gradually replaced by alkyl groups, giving primary, secondary and tertiary amines:

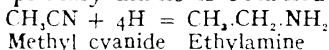
- $\text{NH}_3 + \text{C}_2\text{H}_5\text{I} = \text{NH}_2\text{C}_2\text{H}_5 + \text{HI}$
- $\text{NH}_2\text{C}_2\text{H}_5 + \text{C}_2\text{H}_5\text{I} = \text{C}_2\text{H}_5\text{NH}\text{C}_2\text{H}_5 + \text{HI}$
- $\text{C}_2\text{H}_5\text{NH}\text{C}_2\text{H}_5 + \text{C}_2\text{H}_5\text{I} = (\text{C}_2\text{H}_5)_3\text{N} + \text{HI}$
- $(\text{C}_2\text{H}_5)_3\text{N} + \text{C}_2\text{H}_5\text{I} = (\text{C}_2\text{H}_5)_4\text{N} + \text{I}^-$

As this method gives a mixture of amines, it is not suitable for the preparation of one pure amine. The free bases can be liberated by distilling the mixture with dilute caustic soda and only a partial separation can be effected by fractional distillation.

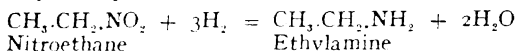
(2) *Hofmann's Method*.—By the action of bromine and caustic potash upon the amide of the monocarboxylic acids containing one carbon atom more than the amine. The bromamide first formed is converted by intramolecular change into an isocyanate, which then breaks up into the amine:



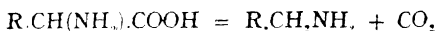
(3) By the reduction of an alkyl cyanide with sodium and alcohol, a primary amine is obtained:



(4) By the reduction of a nitroparaffin with tin and HCl, a primary amine is obtained:



(5) By the breaking down of amino acids by heat or by bacterial action; this may be described as a process of *decarboxylation*:



This *biological method of formation* of amines is very important. Normally, proteins are broken down into amino acids in the intestinal canal. Bacteria acting on these amino acids carry the degradation process further forming amines. Many amines so formed have a marked physiological action; *histamine* (β -iminazole ethylamine), derived from the amino acid histidine (α -amino- β -iminazole propionic acid) is one of them (see p. 248).

General Properties of the Alkylamines.—The lower members are gases with an ammoniacal smell; these are all soluble in water but insoluble in ether or chloroform. The higher members are liquids or solids and their solubility in water gradually decreases with increase in molecular

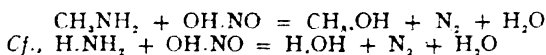
weight. Like ammonia, they are strong *bases* and unite with acids to form salts soluble in water; these salts are however soluble in alcohol, a property which distinguishes them from ammonium salts. With many alkaloidal reagents they give a precipitate, and this should be borne in mind when amines have to be distinguished from alkaloids. Unlike ammonia they are combustible.

Isolation of Naturally Occurring Amines.—Sulphuric acid is added to the solution to make a concentration of 5 per cent; a concentrated solution of pure phosphotungstic acid containing 5 per cent sulphuric acid is then added until there is no more precipitation. The precipitate, which contains all the water-soluble nitrogenous bases together with water-soluble proteins, etc., is filtered over a pump and washed with 5 per cent sulphuric acid. It is then treated with a mixture of 3 volumes of acetone and 4 volumes of water, which dissolves out the phosphotungstates of the bases, and filtered. The bases are then liberated by a saturated solution of barium hydroxide and filtered. The excess of barium is removed from the filtrate by passing CO_2 and the filtrate evaporated in vacuum. The amine or mixture of amines is then purified by special methods.

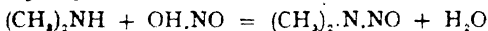
Differentiation of primary, secondary and tertiary amines.

(1) By Nitrous acid—

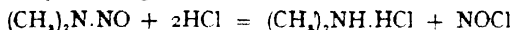
(a) The primary amines react with HNO_2 and yield the corresponding alcohol with evolution of nitrogen (marked effervescence):



(b) The secondary amines give *nitrosamines* which are yellow oily liquids, volatile in steam. No effervescence.



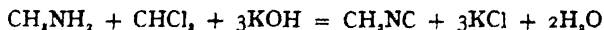
The nitrosamines are converted into the secondary amines by boiling with conc. HCl :



(c) The tertiary amines do not react with HNO_2

(2) By Isocyanide or Carbylamine reaction:

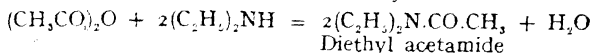
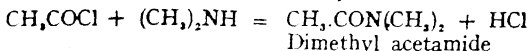
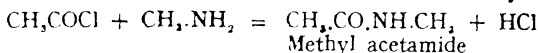
(a) Primary amines, treated with a drop of CHCl_3 and alcoholic potash and heated, form an alkyl (or phenyl) isocyanide with a characteristic disagreeable smell:



(b) Secondary and tertiary amines do not form isocyanides.

(3) *By Acid chlorides and anhydrides:*

They combine with primary and secondary amines to form amides, but have got no action on the tertiary amines.



(4) *By Benzene sulphonic chloride:*

(a) The primary amines react with benzene sulphonic chloride and yield compounds such as $\text{C}_6\text{H}_5\text{SO}_2\text{.NHR}$ which dissolve in aqueous alkalies.

(b) The secondary amines yield compounds of the type of $\text{C}_6\text{H}_5\text{SO}_2\text{.NR}_2$ which are also soluble in alkalies.

(c) The tertiary amines do not react at all.

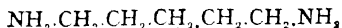
Methylamine, CH_3NH_2 .—This occurs naturally in bone oil and in herring brine. It is a colourless gas, b.p. -6.7° , with an ammoniacal odour and is very easily soluble in water; it is combustible in air thus being easily distinguished from ammonia. With acids it forms salts that are soluble in alcohol; like alkaloids, the halogen salt $\text{CH}_3\text{NH}_2\text{.HCl}$ forms complex platonic chlorides, $(\text{CH}_3\text{NH}_2)_2\text{H}_2\text{PtCl}_6$, and auri-chlorides, $\text{CH}_3\text{NH}_2\text{.HAuCl}_4$; these salts may be used for the detection of the amines.

Diethylamine, $(\text{C}_2\text{H}_5)_2\text{NH}$.—This can be prepared by the action of alcoholic ammonia on ethyl iodide. It is a colourless liquid, b.p. 56° , and is easily soluble in water; it is a stronger base than methylamine and forms salts with acids; it also gives complex platonic chlorides and auri-chlorides. With nitrous acid, it gives the nitrosamine $(\text{C}_2\text{H}_5)_2\text{N.NO}$. It does not give the carbylamine reaction.

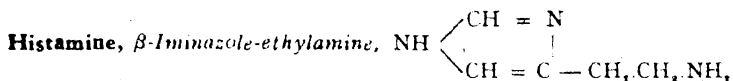
Trimethylamine, $(\text{CH}_3)_3\text{N}$.—This is found naturally in herring brine; it is also obtained by distillation of vinasses (residue from beet sugar manufacture). It is a gas with a fishy ammoniacal odour, b.p. 3.5° . It is a strong base forming salts; it does not give the carbylamine reaction, nor does it react with nitrous acid.

Some Amines of Physiological Importance.

Cadaverine, Pentamethylenediamine,



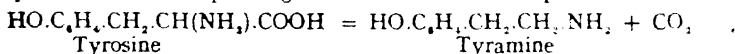
This *diamine*, together with *putrescine* (tetramethylenediamine) has been found in putrid meat. These and other bases are sometimes known as *ptomaines*. Cadaverine can be prepared by the decarboxylation of the amino acid lysine, $\text{NH}_2(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{COOH}$. It is a liquid with an odour of semen, b.p. 178° ; it is a strong base; the platinic chloride forms orange coloured prisms.



This amine is derived from the amino acid histidine by decarboxylation. It is one of the active principles of ergot. It produces temporary rise of blood pressure followed by dilatation of capillaries resulting in fall of blood pressure. It is also formed in damaged tissues producing surgical shock after operation. The formation of weals on the skin is believed to be caused by histamine produced in this tissue when injured. It is known to stimulate the gastric secretion. Histamine is also formed in putrid meat.

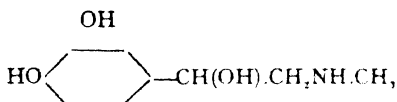
It causes a marked contraction on the isolated uterus even in a dilution of 1 in 25,000,000. It forms colourless needles, m.p. 84° , easily soluble in water but not in ether. On warming with bromine-water, it gives a claret colouration (Knoop's Reaction); this reaction is also given by histidine.

Tyramine, *p*-Hydroxyphenylethylamine, $\text{C}_8\text{H}_{11}\text{NO}$.—This is found in putrid meat, in cheese and in ergot; it can be prepared by heating tyrosine, the corresponding amino acid found in proteins:



Tyrosine is heated under reduced pressure to about 260° ; the tyramine formed is extracted with hot xylene from which it crystallizes out. It forms colourless plates, m.p. 161° . It is soluble in water, hot alcohol, and in hot xylene. It has a marked physiological action and increases blood pressure when injected intravenously. It produces toxic symptoms when absorbed from the intestine in constipation.

Adrenaline, *Suprarenin*, *Epinephrin*, 3:4-Dioxyphenyl-ethanol-methylamine,



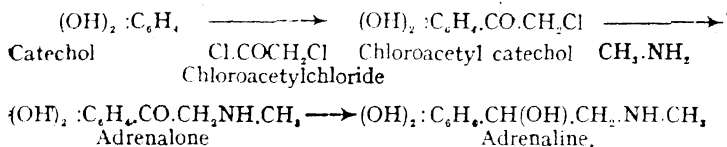
This is the physiological active principle of the suprarenal gland which, even in high dilution, causes a rise of blood pressure when injected.

Preparation.

(1) *From suprarenal glands.*—The minced glands are extracted with absolute alcohol containing some trichloroacetic acid. The filtered

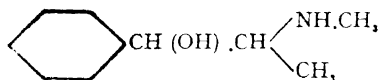
extract is evaporated to a small bulk and filtered. On adding ammonia to the filtrate the base is precipitated; it is washed with alcohol and ether and dried. The compound obtained from this natural source is levorotatory or l-adrenaline.

(2) *Synthetic process*.—Catechol is condensed with chloroacetylchloride and the chloroacetylcatechol formed is treated with methylamine: the adrenalone thus obtained is reduced with aluminium amalgam when racemic adrenaline or dl-adrenaline is obtained; the levorotatory compound is obtained by fractional crystallization of the salt with d-tartaric acid:



Properties and Use.—Adrenaline forms colourless crystals, m.p. 212° . The base is only slightly soluble in water or alcohol. The hydrochloride of the levorotatory base is easily soluble in water and shows the rotation, $[\alpha]_D^{20} = -50^\circ$ (0.12 per cent in water). The laevo compound is about 12 times as active physiologically as the dextro compound, and about twice as active as the dl-compound. The hydrochloride of the laevo base when injected produces a rise of blood pressure. When applied locally to mucous surface it arrests bleeding by constricting the capillaries. It is used in medicine for the treatment of shock, certain forms of heart disease, asthma, hay fever, etc.

Ephedrine, α -Hydroxy- β -methylamino propyl benzene, $\text{C}_{10}\text{H}_{15}\text{NO}$



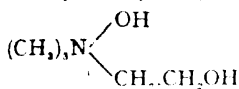
This base is similar to adrenaline in physiological action, but is found only in the vegetable kingdom. It has been isolated from several species of Ephedra growing in China and India (*Ephedra vulgaris* Rich., *Ephedra intermedia* Schrenk, etc.). Its presence in *Sida cordifolia* Linn., a drug used in Ayurvedic medicine, has been established by Ghosh and Dutt. The green twigs of Ephedra may contain from 1 to 2 per cent of total alkaloids, of which more than half consists of ephedrine.

Preparation of Ephedrine.—Coarsely powdered dry green twigs are thoroughly extracted with rectified spirit in a percolator at room temperature. The alcohol is recovered and the semisolid residue is extracted with 1 per cent HCl. The aqueous extract is filtered, made alkaline with ammonia and the alkaloids extracted with chloroform. The chloroform extract is dried with anhydrous sodium sulphate and

the chloroform recovered. The residue is dissolved in alcohol, neutralized with alcoholic HCl and evaporated to dryness. The residue is extracted repeatedly with boiling chloroform and filtered; ephedrine hydrochloride being insoluble in chloroform remains behind in the residue, and it is further purified by recrystallization from alcohol.

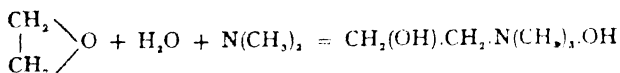
Properties and Use—The base forms colourless crystals, m.p. 43° ; It is soluble in water, alcohol, chloroform and ether; $[\alpha]_D^{20} = 14.95^{\circ}$ (2 per cent in water), -5.08° (3 per cent in absolute alcohol). The hydrochloride forms colourless needles, m.p. 216° , easily soluble in water and alcohol; $[\alpha]_D^{20} = -34^{\circ}$ (5 per cent in water); ephedrine hydrochloride resembles adrenaline in its action but is more prolonged. It is used as a cardiac stimulant and in relieving attacks of asthma.

Choline, Trimethyl- β -hydroxyethyl-ammonium hydroxide,



As mentioned before, it is a constituent of the lipid, lecithin, occurring in egg-yolk, in brain and in other animal tissues. It is found free in semen in quite a large amount (0.51%). It is also found in various plants.

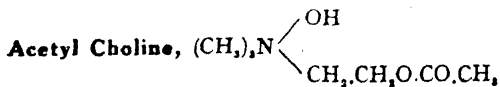
It is synthetically prepared by the action of trimethylamine on ethylene oxide.



It is a hygroscopic liquid, strongly alkaline in reaction and easily soluble in water and alcohol but insoluble in ether. The double compound with HgCl_2 forms colourless needles, m.p. 244° . On heating, choline decomposes into trimethylamine and glycol.

With strong solution of iodine in KI choline gives monoclinic prisms of choline periodide (hexaiodide) which forms the basis of *Florence test* for detection of seminal stains on garments, etc., in medico legal investigation. The crystals are dark brown and look like hæmin crystals.

Carbamylcholine chloride or *Carbachol* (B.P.) is used for urinary retention, paroxysmal tachycardia and hypertension.



This is found in ergot, possesses a marked physiological action, and is used in pharmacological experiments.

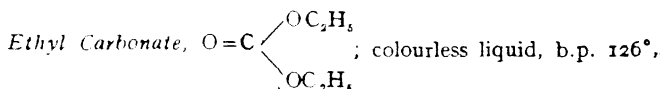
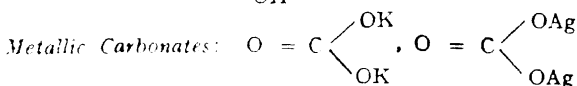
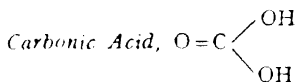
CHAPTER XXI

DERIVATIVES OF CARBONIC ACID

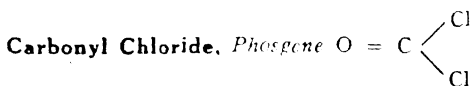
Urethanes, Guanidines, Urea, Ureides, and Purines.

Carbonic acid H_2CO_3 is unstable and is not known in the free state. It behaves as a weak dibasic acid. Its esters, acid chlorides, amides, like the metallic carbonates, are all stable compounds.

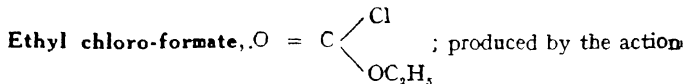
Esters and Chlorides of Carbonic Acid.



formed by the action of ethyl iodide upon silver carbonate.



It is an acid chloride of carbonic acid formed during the decomposition of chloroform; prepared on a large scale by passing CO and Cl_2 through heated charcoal; a colourless gas with a suffocating smell, used as a *poison gas* during the first Great War. It is used in the synthesis of various compounds.



of COCl_2 on ethyl alcohol (see chloroform); used as a reagent for introducing the ester group— COOC_2H_5 into organic compounds.

Amides of Carbonic Acid.

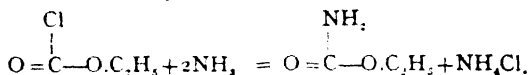
When the OH groups of carbonic acid are gradually replaced by NH_2 groups, we first get *carbamic acid*

$\text{O}=\text{C}\begin{matrix} \text{OH} \\ \text{NH}_2 \end{matrix}$, and finally *carbamide* or urea $\text{O}=\text{C}\begin{matrix} \text{NH}_2 \\ \text{NH}_2 \end{matrix}$. The

alkyl esters of carbamic acid are known as *urethanes*.

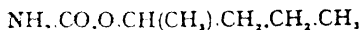
Urethane, Ethyl urethane, Ethyl carbamate,

This is obtained by the action of ammonia on ethyl chloroformate:



Urethane is a colourless crystalline solid, m.p. 50° , easily soluble in water. It distils without decomposition at 184° , but is easily decomposed by NaOH. It is used as a *hypnotic* for children and for experimental animals in pharmacological work.

Hedonal, Methylpropylcarbinol urethane,



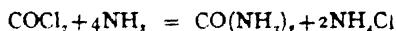
This is stated to possess a stronger hypnotic action than ethyl urethane.

Urea, Carbamide, $\text{O}=\text{C}\begin{matrix} \text{NH}_2 \\ \text{NH}_2 \end{matrix}$

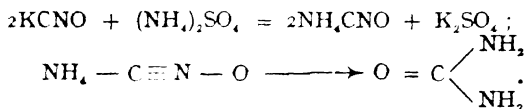
Urea was discovered in 1773 by Rouelle. It was first synthesized by Wöhler in 1828 by evaporating an aqueous solution of ammonium cyanate. This epoch-making discovery of Wöhler clearly demonstrated that organic compounds, identical in every way with those found in the animal organs, could be synthesized in the laboratory.

Occurrence.—Urea is found normally in the urine (1.5—2.5 per cent), in the blood (0.015—0.03 per cent), and in saliva (0.010—0.03 per cent); it is as urea that the greater part of the nitrogen is excreted (about 85 per cent of the total nitrogen) from the human system through the kidney. The average daily excretion of urea for an adult human being is 20-30 grams. In milk it may occur from 0.015 to 0.03 per cent. The blood of shark and dogfish contains as much as 1.7 per cent. It is fairly rich in many moulds and fungi. The urine of tiger contains 6.9 per cent, cat 2.2 per cent and rat 4.5 per cent.

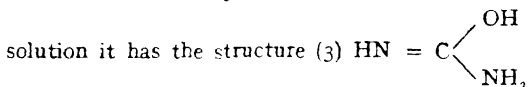
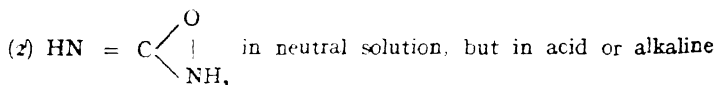
Synthesis of Urea.—(1) By the action of ammonia on carbonyl chloride:



(2) *Wöhler's Method*.—The calculated quantities of potassium cyanate and ammonium sulphate are dissolved in water and evaporated to dryness on the water bath. The urea formed is extracted from the residue with hot alcohol and filtered. On concentrating the alcoholic filtrate and cooling, the urea crystallizes out. It can be purified by recrystallization from hot alcohol. The ammonium cyanate which is formed by double decomposition is converted into urea by molecular rearrangement:



Structure of Urea.—The formation of urea from carbonyl chloride and ammonia as also the action of HNO_2 on urea agrees with the (1) simple carbamide formula $\text{CO}(\text{NH}_2)_2$ for urea. The simple explanation of the reactions in Wöhler's synthesis as well as the carbamide formula suggested by him have, however, been disputed. Werner thinks that normal urea has the cyclic structure,

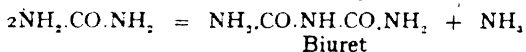


Preparation of Urea. (1) *From Urine*.—Take some urine (500 c.c.) and evaporate down on the water bath to one-fifth of its bulk (100 c.c.). Allow to cool and add about the same amount (100 c.c.) of conc. HNO_3 gradually and with constant stirring, keeping the solution cool; the nitric acid precipitates the urea as crystalline urea nitrate $\text{CO}(\text{NH}_2)_2 \cdot \text{HNO}_3$. Filter off these crystals through glass wool, then make them into a paste with alcohol and treat this with barium carbonate until evolution of CO_2 ceases. The mixture is then dried on the water bath and the urea extracted with hot alcohol. The alcoholic extract is boiled with some animal charcoal to decolorize it and filtered; the filtrate is concentrated on the water bath and on cooling, urea separates out; it is purified further by recrystallization from hot alcohol.

(2) *On a large scale*, urea is prepared for its use as fertilizer by the action of liquid carbon dioxide upon liquid ammonia under pressure.

Properties and Reactions.—Urea crystallizes in long colourless prisms, m.p. 132° . It is easily soluble in water, methyl alcohol and glycerol, fairly soluble in alcohol and acetone but insoluble in ether, chloroform and benzene. It is neutral in reaction but acts as a weak base forming crystalline salts with strong acids like nitric acid, $\text{CO}(\text{NH}_2)_2 \cdot \text{HNO}_3$, and oxalic acid, $[\text{CO}(\text{NH}_2)_2]_2 \cdot \text{C}_2\text{H}_2\text{O}_4$.

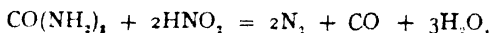
Urea melts at 132° , and when heated to about 155° it evolves ammonia and forms the compound known as biuret:



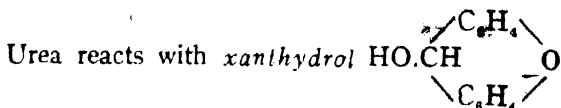
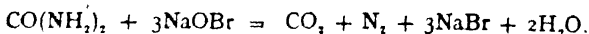
The biuret found in the melted mass can be extracted with water; it crystallizes from water in needles, m.p. 193° . A solution of biuret made alkaline with a dilute solution of NaOH gives a violet colour with a dilute solution of copper sulphate. This is known as the *biuret reaction* and is given by compounds having at least two — CO — NH — groups in the molecule; it is thus given by proteins. When heated further (about 170°), urea forms *cyanuric acid* and ammonia:



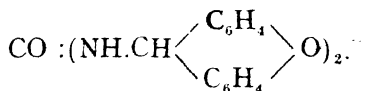
Urea is decomposed by nitrous acid with the evolution of nitrogen and carbon dioxide:



A solution of *sodium hypochlorite* or *hypobromite* also decomposes urea into carbon dioxide and nitrogen. The usual method of estimation of urea is based upon this reaction:

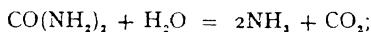


in presence of an acid like acetic acid giving an insoluble crystalline compound *dixanthyl urea*



This reaction is very delicate and is used in micro-detection and micro-estimation of urea.

Urease, a specific enzyme for urea found in *soya beans* (seeds of *Soya hispida* Mnch.), *Arhar dal* (*Cajanus indicus*), and in other seeds and in certain bacteria (*Micrococcus ureae*) hydrolyzes urea to ammonia and carbon dioxide:



this reaction is utilized in detection as well as in the estimation of urea (see later). The strong smell of ammonia in public latrines is due to this reaction—hydrolysis of urea by urease from bacteria.

With mercuric nitrate, urea gives a curdy white ppt. of a basic compound of mercuric nitrate and urea, $[\text{CO}(\text{NH}_2)_2]_2 \cdot \text{Hg}(\text{NO}_3)_2 \cdot 3\text{HgO}$. This reaction is also a delicate test for urea.

Estimation of Urea.—This is important for testing *renal efficiency* and in other biochemical problems. The hypobromite method is simple and rapid and is commonly used for the urine. The urease method gives accurate results and is used in estimating small quantities of urea, such as that found in blood, cerebrospinal fluid, etc.

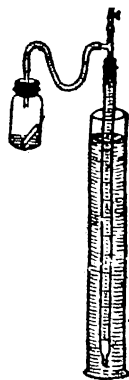


Fig. 37

(1) *Hypobromite Method.* (a) An alkaline solution of hypobromite, as stated before, reacts with urea and gives off nitrogen and carbon dioxide, and it can be found by calculation that one gram of urea corresponds to 373 c.c. of nitrogen gas at N.T.P. (60 grams of $\text{CO}(\text{NH}_2)_2$ yields 22.4 litres of N_2). For the estimation of urea in urine, about 25 c.c. of a freshly prepared solution of sodium hypobromite, prepared by dissolving 10 grams of NaOH in 25 c.c. of water and adding 2.5 c.c. of bromine, are taken in a small bottle provided with a rubber stopper and connected by a rubber tube to a gas burette almost filled with water (Fig. 37). Five c.c. of the sample of

urine are taken in a small tube and placed inside the bottle without allowing the hypobromite to come in contact with the urine. After making the connections air-tight, the volume of air in the burette is read at atmospheric pressure. The bottle is then tilted to allow the urine to come in contact with the hypobromite. The carbon dioxide is absorbed by the alkali and nitrogen is liberated. When the gas attains the room temperature, the volume is read at atmospheric pressure and the increase in volume, due to nitrogen, is reduced to N.T.P. The percentage of urea can be calculated from this.

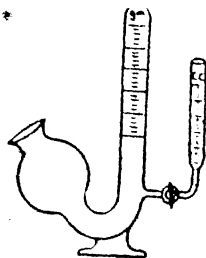


Fig. 38

(b) *By Doremus apparatus.*—This is the quickest method for clinical purpose. The apparatus (Fig. 38) is filled with alkaline sodium hypobromite and a known volume of the urine is allowed to flow in from the graduated side tube. The nitrogen is collected in the main tube which is graduated in grams

of urea from which the percentage is easily calculated.

Owing to secondary reactions (e.g., formation of cyanate, hydrazine, etc.) caused by hypobromite, these methods are never accurate and give results which are about 7 per cent below the theoretical amount.

(2) *Urease Method.*—As mentioned above, the enzyme *urease*

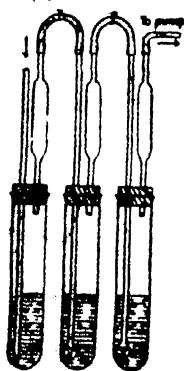
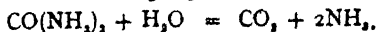


Fig. 39

decomposes urea with the formation of ammonia and carbon dioxide, and the amount of urea can be calculated from the amount of ammonia formed. The solution of urea (approx. 5 c.c. of a 2 per cent solution) is taken in the middle one of a series of three test tubes connected together by tubes (Fig. 39). About 1 gram of powdered soya bean is added to the urea solution and the test tube is kept at about 40° in the water bath for about 30 minutes. The first tube, which acts as a washer, contains some dilute sulphuric acid and the third tube contains a known volume of N/10 sulphuric acid with 2 or 3 drops of an indicator. The test tubes are connected with a pump and a slow current of air is drawn in. After ten minutes, about one gram of anhydrous sodium carbonate is put into the middle test tube,

quickly mixed by gentle shaking and connected again with the pump. The sodium carbonate liberates any ammonia which may be fixed as ammonium salt. By back titration with a standard alkali one can ascertain the amount of ammonia evolved from the urea, one c.c. of N/10 alkali being equivalent to 0.003 g. of urea as can be calculated from the following equation:



Amidine Derivatives of Carbonic Acid.

The *amidines* contain the monovalent group $\text{—C} \begin{array}{l} \diagup \text{NH} \\ \diagdown \text{NH}_2 \end{array}$

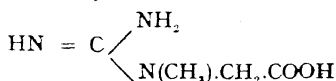
These are fairly strong bases and form stable salts with acids. They are, however, easily hydrolyzed by acids or alkalis, thus differing from amines.

When the oxygen atom of urea is replaced by the imino group ($=\text{NH}$), we get the amidine known as *guanidine*, $\text{HN} = \text{C} \begin{array}{l} \diagup \text{NH}_2 \\ \diagdown \text{NH}_2 \end{array}$. Some guanidine derivatives, such as creatine, creatinine, arginine, etc., occur in animal tissues.

Guanidine, *Imino carbamide*, *Imido urea*, $\text{HN} = \text{C} \begin{array}{l} \diagup \text{NH}_2 \\ \diagdown \text{NH}_2 \end{array}$

This has been found in certain plants and can also be obtained from some proteins. It is prepared by heating ammonium thiocyanate to about 180° . It is a very deliquescent crystalline substance, easily soluble in water and alcohol. It is a strong base, absorbs CO_2 from the air, and is toxic to animals. It is hydrolyzed by alkalis to urea and ammonia: $\text{NH}_2\text{C}(\text{NH}_2)_2 + \text{H}_2\text{O} = \text{CO}(\text{NH}_2)_2 + \text{NH}_3$.

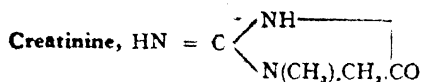
Creatine, α -Methyl guanidine acetic acid,



It is an important and constant constituent of muscles (*kreatos*-of meat) of all vertebrates and is also found in other tissues. Thus, the striped muscle of mammals may contain up to 0.6 per cent, and blood may contain about 0.01 per cent of creatine. It is, however, generally absent from the normal male (adult) human urine, although it is found in the urine of women and young children. It is said to play a part in the metabolism of muscle contraction.

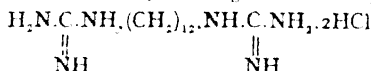
It can be prepared by extracting muscle with hot water and treating the filtered extract with lead acetate until there is no more precipitation. The filtrate is treated with H_2S and the filtered liquid is concentrated to a small bulk and left in an ice chest to crystallize. The crystals, which can be recrystallized from hot water, are washed with 88 per cent alcohol and dried. It is found in the meat extract to the extent of about 6 per cent.

Creatine crystallizes from water in colourless prisms with one molecule of water of crystallization, which is lost when heated to 100° . It is easily soluble in hot water but less so in cold; it is insoluble in alcohol and ether, and is neutral in reaction. It has a faint reducing action. By heating or by treatment with a dehydrating agent creatine becomes converted into creatinine.

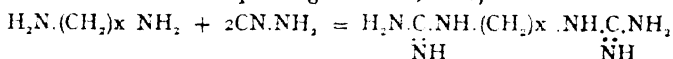


This is the *anhydride of creatine*, and occurs in all mammalian urines and in blood as a *normal* constituent. The average daily excretion in a healthy adult is about 1 gram. It forms colourless crystals, only slightly soluble in cold water and alcohol but readily in hot. It is a strong base and the aqueous solution has a slightly alkaline reaction. It reduces solutions of Cu, Ag, Hg and picric acid. The reduction of Fehling's solution by urine containing excess of creatinine may thus be mistaken for a reducing sugar. If a few drops of a freshly prepared solution of sodium nitroprusside be added to a solution of creatinine and followed by a few drops of dilute solution of KOH, a ruby red colour is produced which soon passes to yellow. On warming and then acidulating with acetic acid a green colour develops which soon changes to blue. Phosphomolybdic acid solution produces a yellow crystalline precipitate.

Synthalin B, *Dodecamethylene diguanidine dihydrochloride*,



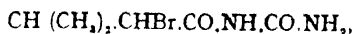
This compound and the decamethylene diguanidine bihydrochloride, known as *Synthalin*, can be prepared by the action of cyanamide on the corresponding diamines; thus,



These guanidine derivatives possess the property of reducing blood sugar when taken orally. They have, therefore, been suggested as oral substitutes for *insulin* for treatment of glycosuria.

UREIDES

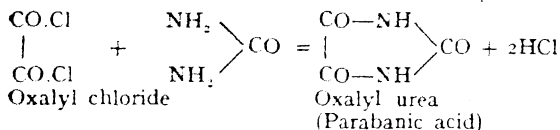
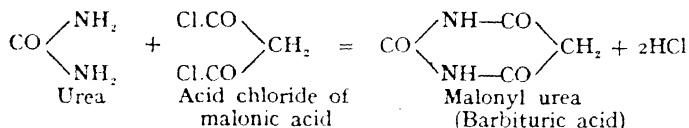
Simple Ureides.—The simple ureides are acyl derivatives of urea formed by the action of acid chlorides or acid anhydrides on urea. Thus, *acetyl urea*, $\text{H}_2\text{N.CO.NH.COCH}_3$, formed by the action of acetyl chloride or acetic anhydride on urea, is a simple ureide. *Bromural* or α -Bromo-isovaleryl urea,



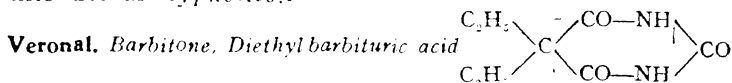
another simple ureide, is used in medicine as a sedative and hypnotic.

Cyclic Ureides.—These are the products of reaction of the acid chlorides of dibasic acids with urea. Both acyl groups take part in the reaction with elimination of 2 mols.

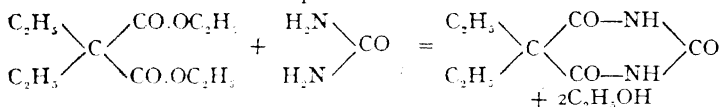
of HCl and cyclic derivatives are formed. Thus malonyl urea or *barbituric acid* is formed as follows:



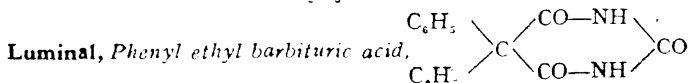
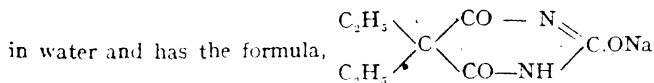
Various derivatives of barbituric acid, known as *barbiturates*, have been synthesized and many have come into use as *hypnotics*.



This may be obtained by condensing the diethyl derivative of malonic acid with urea in presence of sodium ethoxide:



It is a white crystalline powder, m.p. 191° . It is very slightly soluble in water but is soluble in alkaline solutions as well as in alcohol, ether and chloroform. It has a powerful hypnotic action. The sodium salt of veronal, known as *veronal-sodium* or *medinal*, is easily soluble



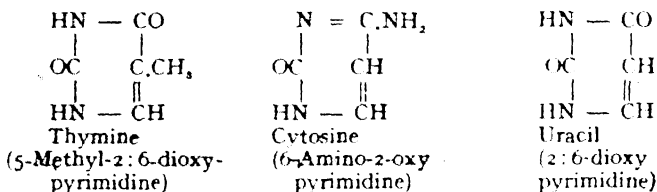
This is a white powder with a slightly bitter taste. It is almost insoluble in water but is soluble in alcohol, ether and chloroform. It is also a powerful hypnotic. Its sodium salt, which is easily soluble in water, is also used in medicine.

PYRIMIDINES

These are *cyclic ureides* obtained from urea and acids with three carbon atoms, and come more properly under *heterocyclic compounds* (see Chapter 26). The parent substance, *pyrimidine* $\text{C}_4\text{H}_4\text{N}_2$, has been prepared synthetically and has the following structure:

- (1) $\text{N} = \text{CH}$ (6) These pyrimidines are found in the proteins of
 (2) $\begin{array}{c} | \\ \text{CH} \end{array} \begin{array}{c} | \\ \text{CH} \end{array}$ (5) cell-nucleus (in nucleic acids), and are therefore
 (3) $\begin{array}{c} || \\ \text{N} - \text{CH} \end{array}$ (4) of physiological significance.

Some of the common pyrimidines found in proteins are shown below:

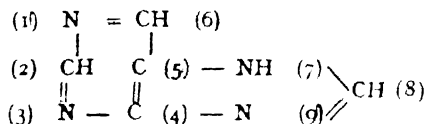


Complex Cyclic Diureides, Purines.

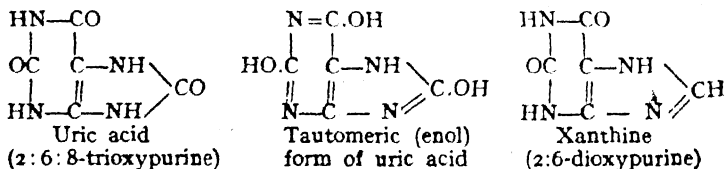
These diureides contain two urea residues,

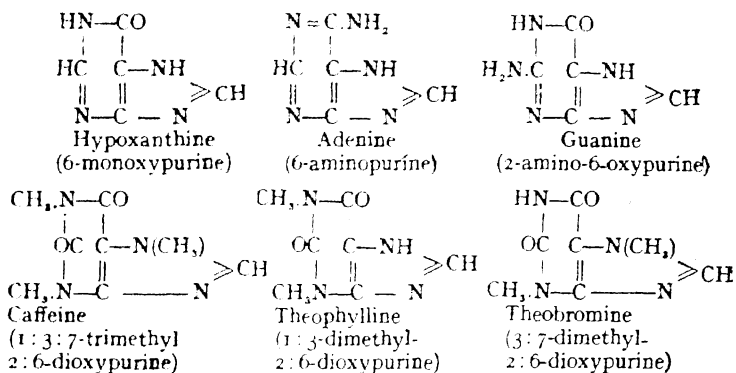


in the molecule. The parent substance *purine* has been obtained synthetically and has the following structure:



It is a colourless crystalline compound, m.p. 216° , with amphoteric reaction, readily soluble in H_2O . It does not occur in nature, but several members of the purine group occur both in the animal and vegetable kingdom and are of some physiological importance. Those occurring mainly in animal tissues, are uric acid, xanthine, hypoxanthine, adenine and guanine, and those commonly occurring in the vegetable kingdom are caffeine, theobromine, theophylline, etc. The structural formulæ of these purines are shown below:





Uric Acid, 2:6:8-Trioxypurine, $\text{C}_5\text{H}_4\text{O}_3\text{N}_4$

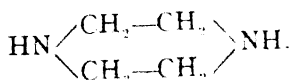
This was discovered in urinary calculi by Scheele in 1776. In normal human urine, it occurs to the extent of 0.5 to 1.5 grams in the 24 hours' sample, but the amount varies with the diet. It is derived from the breaking down of nucleoproteins in the organism. It occurs to a much larger extent in the excrements of birds and reptiles. It occurs in human blood to the extent of about 2-3 milligrams per 100 c.c.; the amount is increased in gout, nephritis and lead-poisoning.

Preparation. (1) *From Urine.*—Urine is treated with one-tenth of its volume of conc. HCl and allowed to stand in a cool place for about 24 hours. The reddish brown crystals of uric acid, which mostly adhere to the sides of the vessel, are removed and purified by conversion into an alkali salt and reprecipitation with HCl.

(2) *From Guano.*—Guano, a deposit of bird's excrement consisting chiefly of ammonium urate found in certain parts of South America, forms a cheap source of uric acid. It is boiled with dilute sodium carbonate until the evolution of ammonia has ceased and then filtered. The filtrate is treated with dilute HCl when uric acid is precipitated. It is purified by repeating the process and drying.

Properties, Reactions and Tests.—The uric acid which separates from urine is pigmented yellow or brown and assumes various shapes such as whetstones, dumb-bells,

sheaves, etc. But the pure acid is a colourless, tasteless, crystalline powder, insoluble in cold water, sparingly so in hot water and insoluble in alcohol and ether. It dissolves in glycerol and in solutions of certain alkali salts such as sodium phosphate, etc. It does not possess any COOH group but nevertheless it behaves as a weak dibasic acid and usually forms acid salts. The neutral salts are more soluble. The most soluble salt is the *lithium* salt and the salt with the organic base *piperazine*.



In gouty subjects, the acid salt of uric acid is deposited in the affected joint.

When heated, uric acid is decomposed into urea, cyanuric acid, hydrocyanic acid and ammonium carbonate. On evaporating a little uric acid with dilute nitric acid to dryness, a beautiful orange colour is developed and on treating the residue with a drop of ammonia, a purple colour (due to ammonium purpurate or *murexide*) is obtained, and if a drop of dilute caustic soda is now added the colour changes to blue-violet. This is known as the *murexide test*. If uric acid is dissolved in sodium carbonate and the solution is poured into a filter paper moistened with silver nitrate solution, there is a black stain due to reduced silver. This is known as *Schiff's test*. Uric acid or urates reduce Fehling's solution on prolonged boiling, an important point to remember when testing urine for sugar. It decolorizes KMnO_4 solution both in acid and alkaline media. Standard KMnO_4 solution with strong H_2SO_4 is therefore used in estimating uric acid. Very small quantities of uric acid can be *estimated* colorimetrically by the blue colour developed by Folin's phosphotungstic acid reagent.

The hydrogen atom of the imino group in $-\text{CO.NH.CO}-$ is acidic in nature and is replaceable by metals. The hydrogen atoms of the OH groups in the enol form are also replaceable by metals and neutral salts are formed when both the H atoms are replaced.

Xanthine, 2:6-Dioxypurine, $\text{C}_5\text{H}_4\text{O}_2\text{N}_4$.—This occurs in small quantities in urine and in animal tissues. It is also found in small

quantities in yeast, beet-root, tea, etc. It is a colourless powder, very slightly soluble in water and insoluble in alcohol and ether.

Hypoxanthine, *6-Monoxypurine*, $C_5H_4ON_4$.—This occurs in small quantities in urine and in animal tissues. It is also found in yeast, in some plant seeds, etc. It is a colourless microcrystalline powder, slightly soluble in water but insoluble in alcohol.

Adenine, *6-Aminopurine*, $C_5H_5N_5$.—This occurs in the pancreas and other tissues. It is also found in yeast, beet-root, tea, etc. It forms colourless crystals, soluble with difficulty in cold water but more easily in hot.

Guanine, *2-Amino-6-oxypurine*, $C_5H_5ON_3$.—This occurs in the animal tissues and is a constituent of nucleic acid. It is also found in small quantities in yeast, sugar-cane, etc. It is a colourless powder, insoluble in water and alcohol. It is soluble in mineral acids or alkalies.

Caffeine, *1:3:7-Trimethyl-2:6-dioxypurine*, $C_8H_{10}O_2N_4$.

Occurrence.—It occurs to the extent of 1 to 4.8 per cent in tea leaves, 1 to 1.5 per cent in coffee beans, 2.5 to 3.5 per cent in kola nuts, etc.

Preparation.—Caffeine is generally prepared on a large scale from damaged tea, waste tea or tea dust. *In the laboratory*, it can be easily prepared by digesting tea with about 5 times the bulk of boiling water and filtering through fine muslin. The residue is washed with some more boiling water and the filtrate is treated with a solution of basic lead acetate until there is no more precipitate, and filtered hot. The proteins, tannins and colouring matter are thus removed. The excess of lead is precipitated by adding dilute sulphuric acid. The filtrate is then concentrated to about one-third the bulk after the addition of animal charcoal. The concentrated solution is filtered, cooled, and extracted 2 or 3 times with chloroform. The chloroform is recovered and the residue recrystallized from hot water.

Properties, Reactions and Uses.—Caffeine crystallizes from water in colourless, thin, silky needles with a molecule of water of crystallization, which is lost at 100° or by keeping over conc. H_2SO_4 . The anhydrous substance melts at 235° but it begins to sublime at about 176° . It has a bitter taste like alkaloids. It is fairly soluble in chloroform (1 in 8 at 25°), water (1 in 46 at 25°) and alcohol (1 in 53 at 25°) but very slightly soluble in ether. It is a weak base giving

a neutral solution in water and its salts are hydrolyzed by excess of water.

Caffeine does not give any precipitate with Mayer's reagent (potassium mercuric iodide), and this distinguishes it from most alkaloids. It is, however, precipitated by Wagner's reagent (I in KI) in the presence of HCl. Caffeine gives the murexide test which is carried out as follows: It is moistened with bromine water and the solution evaporated to dryness on a water bath; the orange coloured residue becomes red on further heating and is turned purple on adding a drop of ammonia.

Tea and coffee are used as stimulant drinks. Caffeine itself is largely used in medicine as a cardiac stimulant and also as a diuretic.

Theobromine, 3:7-Dimethyl-2:6-dioxypurine, $C_7H_8O_2N_4$.

This purine is found in coca beans and in small amounts in kola nuts and in tea leaves. It forms microscopic crystals, m.p. 330° (sublimes at 290°); very sparingly soluble in cold water, but moderately soluble in hot water and in boiling chloroform. It gives the murexide test but no precipitate with Mayer's reagent. The soluble salts are used in medicine, chiefly as diuretic.

Theophylline 1:3 Dimethyl-2:6-dioxypurine, $C_7H_8O_2N_4$.

This purine is found in small quantities in tea leaves. It forms colourless crystals, sparingly soluble in cold water but readily in hot water. Its medicinal use is similar to that of theobromine.

PART III

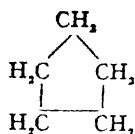
CYCLIC OR CLOSED-CHAIN COMPOUNDS

AROMATIC COMPOUNDS

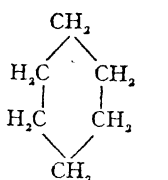
CHAPTER XXII

THE BENZENE HYDROCARBONS

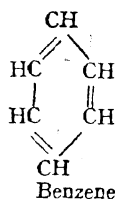
Cyclic Compounds—In addition to the aliphatic compounds which are open-chain compounds, there are a great variety of substances having ring structure or a closed chain of carbon atoms. These are called *cyclic* compounds and are divided into two classes—*homocyclic*, having only carbon atoms in the ring, and *heterocyclic* containing carbon atoms as well as nitrogen, sulphur, etc. The homocyclic compounds are again divided into two groups, namely, (1) *alicyclic compounds* represented by cyclopentane, cyclohexane, etc., which resemble aliphatic compounds in their properties, and (2) *aromatic compounds* having a peculiar type of unsaturation as exhibited in the structure of the first member, benzene, as shown here.



Cyclopentane

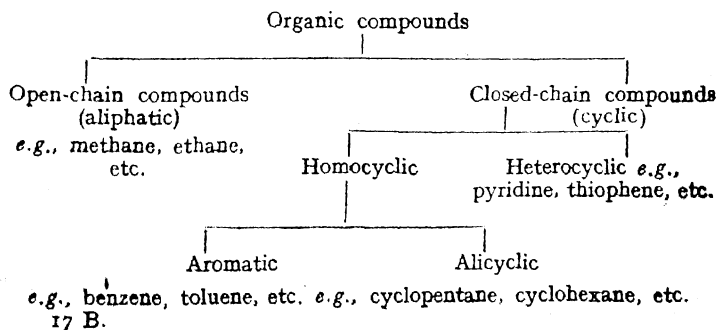


Cyclohexane



Benzene

We may, therefore, state as follows:



Just like the aliphatic hydrocarbons, aromatic hydrocarbons also give alcohols, aldehydes, ketones, acids, etc., whose general properties are similar to those of the aliphatic groups. During the last half a century the study of the aromatic compounds has created great interest on account of their industrial possibilities. Aromatic hydrocarbons of commercial importance occur in the distillation products of coal tar described below.

N.B. The name aromatic originated from the fact that in the earlier stages of our chemical knowledge, these compounds were considered to be derived from substances such as oil of turpentine, oil of cloves, cinnamon oil, resins, etc., which possessed an aromatic odour. This idea is, however, no longer tenable and aromatic compounds are now almost synonymous with *derivatives of benzene*.

General Properties of Aromatic Compounds

In many ways the aromatic compounds resemble the *aliphatic* ones, while in other ways they differ considerably. They have a much *higher carbon content* than the aliphatic and so burn with a smoky flame. They possess a *high molecular weight* and are more often *crystalline*. Their reaction with nitric acid and sulphuric acid are very distinctive. When treated with concentrated nitric acid they readily yield *nitro-compounds*, and with conc. sulphuric acid they give *sulphonic acids* whereas the aliphatic compounds are scarcely attacked under the same conditions. The *halogen atoms* attached to an aromatic ring are not easily replaced by amino or hydroxyl groups as they are in the aliphatic compounds. The aromatic *amines* are less basic than the aliphatic amines and the *phenols* are more acidic than the corresponding aliphatic compounds due to the negative nature of the phenyl radical— C_6H_5 . The reduction of an aliphatic *nitro-compound* would give the corresponding amine but in the case of an aromatic nitro compound we get intermediate products like *azo*- and *azoxy-compounds*. The amino groups of aliphatic or aromatic *amines* are replaced by hydroxyl groups when treated with nitrous acid, but the aromatic amines form intermediate *diazo compounds* while the aliphatic amines scarcely do so.

Dry Distillation of Coal

Coal is heated in large fire-clay retorts at about $1000^{\circ}C$ out of contact with air. The products of decomposition are

first passed through large vertical air-condensers for condensing the *coal tar* and the aqueous liquid, known as *gas liquor*, which collect in tanks. The gases formed are freed from further quantities of tar and gas liquor by passing through air- or water-cooled chambers. They are next freed from hydrocyanic acid, sulphuretted hydrogen, etc., by passing through special washing chambers. The purified *coal gas* is collected in large gas holders over water, from where it is distributed through pipes. The *yield of coal tar* by this method (*high temperature distillation or carbonization*) is about 6 per cent of the weight of coal, and the yield of coal gas is about 10,000 cubic feet per ton of coal or about 17 per cent of the weight of coal. The residue left behind in the retorts is known as *coke*.

The *composition of coal gas* varies with the nature of the coal and the temperature of distillation. The approximate composition of an ordinary sample of purified coal gas may be taken as follows: (1) Non-luminous constituents such as *hydrogen* 50 per cent, *methane* 32 per cent, *carbon monoxide* 8 per cent, etc.; (2) luminous constituents (such as *ethylene*, *acetylene*, *benzene*, *naphthalene*, etc.) 4 per cent; (3) other constituents such as *nitrogen* 4 per cent, *carbon dioxide*, traces of *ammonia* and *sulphuretted hydrogen* 2 per cent.

The hydrocyanic acid absorbed in washing chambers serves as a source of potassium ferrocyanide and other *cyanogen compounds*. The gas liquor, which contains a large quantity of ammonia, is a cheap source of various ammonium salts, ammonium sulphate being largely used as a fertilizer. The yield of *ammonia as sulphate* is about 25 pounds per ton of coal.

Distillation of Coal Tar

As stated above, the coal tar forms about 6 per cent of the weight of coal used, but the amount may vary with the nature of the coal and the temperature of distillation. For instance, at *low temperature carbonization* (about 500°C) the amounts of gas and ammonia are reduced while the amount of tar increases. The tar is separated from the aqueous gas liquor and submitted to *fractional distillation* in large iron stills connected with long iron condensers. The distillate

consists of the following four fractions, and the yield is from one ton of the tar:

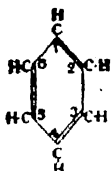
- I. *Light Oil* or Crude Naphtha
B.P. up to 170° ; sp. gr. 0.92, hence 'light' oil; yield about 12 gallons, i.e., about 5 per cent; contains benzene, toluene, xylene, etc., as well as small amounts of pyridine, phenol, etc.
- II. *Middle Oil* or Carbolic Oil
B.P. 170° to 230° ; sp. gr. 1.01; yield about 20 gallons, i.e., about 9 per cent; contains naphthalene, phenol, cresols, etc.
- III. *Heavy Oil* or Creosote Oil
B.P. 230° to 270° ; sp. gr. 1.04; yield about 17 gallons, i.e., about 8 per cent; contains cresols and small amounts of naphthalene; used as a preservative for timber.
- IV. *Anthracene Oil* or Green Oil
B.P. 270° to 400° ; sp. gr. 1.10; yield about 38 gallons, i.e., about 19 per cent; contains mainly anthracene, and some phenanthrene; used for preparing various dyes.
- V. *Pitch*
This is the residue remaining in the still; yield about 11 cwt. i.e., about 55 per cent; used for making briquettes from coal dust, for preparing black varnishes and also in making roads.

Fractional Distillation of Light Oil

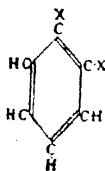
The light oil is shaken with concentrated sulphuric acid to remove basic substances such as pyridine, etc., and then with dilute caustic soda to remove acidic substances such as phenol, etc. It is next washed with water and submitted to fractional distillation. The four fractions collected are: (1) up to 70° , (2) between 70° and 140° , (3) between 140° and 170° and (4) over 170° . The second fraction on further fractionation yields mainly benzene, toluene and xylene. The higher hydrocarbons distilling after xylene, either from the second fraction or from higher fractions, are known as *solvent naphtha* which used in various industries and particularly as a solvent for rubber.

Constitution of Benzene

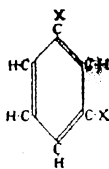
The first member of the aromatic hydrocarbons is benzene. The percentage composition and vapour density determinations show that the molecular formula is C_6H_6 , which obviously shows that it is an unsaturated compound. Any structural formula for benzene must explain the following facts: (1) It gives one and only one mono-derivative, hence the structure must be symmetrical, (2) it gives three di-derivatives. These facts are fully accounted for by ascribing to it the following *hexagonal formula with alternate double linkages* as suggested by Kekulé in 1865. As the structure is symmetrical, it accounts for the *stability* of benzene towards chemical agents and it also accounts for the existence of only one *mono-derivative*, since all the six hydrogen atoms are symmetrically placed with respect to one another; further, it explains the existence of *only three di-derivatives*.



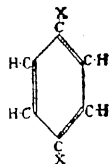
With di-derivatives, three isomerides are possible, namely the 1:2 position or *ortho* compound, the 1:3 position or *meta* compound and the 1:4 position or the *para* compound. These isomers are known as *position isomers*. The position 1:2 is similar to position 1:6 since they



Ortho (1:2)

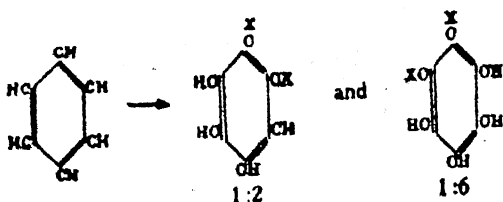


Meta (1:3)

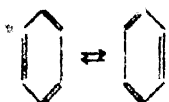


Para (1:4)

are adjacent, and the position 1:3 is similar to position 1:5 since they are *alternate* in the ring. But here again a difficulty arises. The position 1:2 with a *double bond* between the carbon atoms does not appear to be identical with the position 1:6 which has only a single bond. One may, therefore, expect that there would be two isomeric di-derivatives of the types shown below:



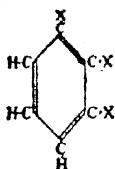
We know, however, that such isomerides do not exist, and this objection was met by Kekulé by the suggestion that the double linkages were continually oscillating between the positions as represented in the diagram.



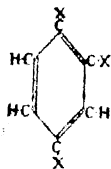
Baeyer suggested that the fourth valency of each carbon atom is directed towards the centre of the hexagon, benzene having a *centric formula*. Various other structures have also been suggested but the majority of chemists prefer the formula suggested by Kekulé.



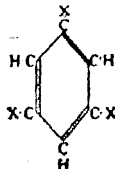
As regards the *tri-derivatives* of benzene, three isomerides are possible, namely, 1:2:3 or *adjacent* (vicinal), 1:2:4 or *unsymmetrical* and 1:3:5 or *symmetrical*, and these are shown below where X represents the substituent!



Adjacent
1:2:3



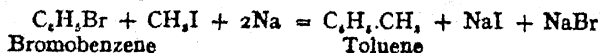
Unsymmetrical
1:2:4



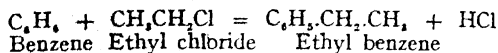
Symmetrical
1:3:5

General Methods of Synthesizing Benzene Hydrocarbons

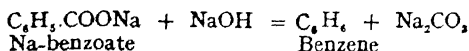
(1) *Fittig's Method*.—By the action of a brominated benzene hydrocarbon and an alkyl bromide upon metallic sodium in dry ether:



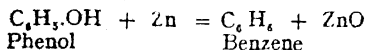
(2) *Friedel and Crafts' Method.*— By the action of alkyl halides on aromatic hydrocarbons in the presence of anhydrous aluminium chloride:



(3) By heating the sodium salts of aromatic acids with soda lime:



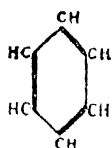
(4) By distilling phenols with zinc dust:



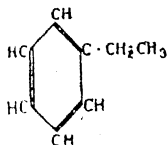
General Properties of Benzene Hydrocarbons

The benzene hydrocarbons are mostly all colourless mobile liquids, with a peculiar odour, insoluble in water but soluble in alcohol and ether. They are volatile with steam. They are as a rule readily nitrated and sulphonated. On oxidation, the *side chains* are readily oxidized to carboxylic acids, and *where more than one alkyl group is present the higher is usually attacked first.*

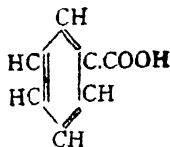
Usually by the term *benzene nucleus* is understood the group of six carbon atoms with its attendant six hydrogen atoms, and the group C_6H_5 — is known as the *phenyl radical*. Where a hydrogen atom of benzene is replaced by a radical, the arrangement is spoken of as a *side chain*. It is important to remember that although the property of an aliphatic side chain is modified by its attachment to an aromatic nucleus, its general chemical behaviour very closely resembles the reactions of an aliphatic compound.



Benzene
(nucleus)



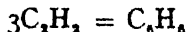
Ethyl benzene
(nucleus with an
aliphatic side chain)



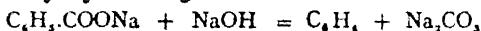
Side chain
oxidized

Benzene, Benzol, C_6H_6 **Synthesis**

(1) By passing acetylene through a red-hot tube:



(2) Pure benzene can be prepared in small quantities in the laboratory by heating sodium benzoate with soda lime.



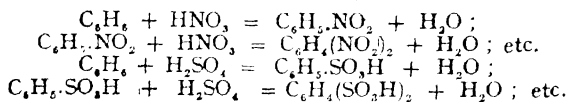
Preparation.—Benzene is prepared on a large scale from the *light oil fraction* obtained by the distillation of coal tar. The *second fraction* obtained by the distillation of light oil (see above) is refractionated and the distillate between 80° and 85° is collected. This is again fractionated carefully until pure benzene is obtained.

Properties, Uses and Reactions.—Benzene is a colourless mobile liquid with a characteristic smell. It boils at 80° and solidifies at 5.4° . It has a sp. gr. of 0.8799 at 20° . It is highly inflammable and burns with a luminous, smoky flame. It is insoluble in water but mixes with methyl alcohol, ethyl alcohol, acetone, ether and glacial acetic acid.

It is a good solvent for resins, fats and oils and also for inorganic substances like iodine, phosphorus and sulphur. It is used both as a solvent and also as the starting material for the preparation of various synthetic aromatic compounds used as dyes and drugs.

Benzene is fairly stable towards mild oxidizing and reducing agents. It does not decolorize bromine-water although it contains three double bonds. In the presence of a suitable catalyst, however, it is reduced by hydrogen to *hexahydrobenzene* C_6H_{12} . Iodine has no direct action upon benzene. Chlorine or bromine acts upon benzene in *direct sunlight* giving *additive products* like benzene hexachloride $C_6H_4Cl_6$ and benzene hexabromide $C_6H_4Br_6$. In *diffused daylight*, especially in presence of a carrier like iodine or antimony trichloride, chlorine gives *substitution products* such as monochlorobenzene C_6H_5Cl , dichlorobenzene $C_6H_4Cl_2$ and trichlorobenzene $C_6H_3Cl_3$; and bromine has a similar action. Caustic alkalies have no action upon benzene. Dilute nitric

acid or sulphuric acid does not react with benzene, but concentrated nitric or concentrated sulphuric acid forms *substitution products*. The hydrogen atoms of benzene are gradually replaced by nitro and sulphonic acid groups, giving mono-, di- or tri-derivatives:



Homologues of Benzene

Benzene is the first member of a homologous series with the general formula $\text{C}_n\text{H}_{2n-6}$, and they are formed by the substitution of hydrogen atoms by alkyl groups. Most of these occur in the light oil fraction obtained from coal tar distillation; of these toluene and xylene are important owing to their use in biochemical and histological work.

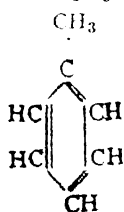
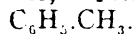
C_6H_6 Benzene.

$\text{C}_6\text{H}_5\text{CH}_3$ Methyl benzene or Toluene.

$\text{C}_6\text{H}_4(\text{CH}_3)_2$ Dimethyl benzenes or Xylenes (o-, m-, p-).

$\text{C}_6\text{H}_3(\text{CH}_3)_3$ Trimethyl benzenes, *e.g.*, mesitylene (1:3:5), pseudocumene (1:2:4), and hemimellitene (1:2:3).

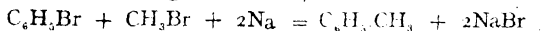
Toluene, Toluol, Methyl benzene, Phenyl methane,



Occurrence.—It is obtained by the dry distillation of Balsam of Tolu, whence the name. Balsam of Tolu is the exudate obtained from the trunk of the tree *Myroxylon toluiferum*, Hb. & Kth. Toluene occurs in the light oil fraction of coal tar distillate from which it is usually prepared by fractional distillation.

Synthesis.

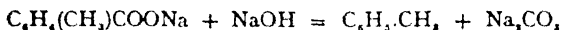
(1) By treating bromobenzene and methyl bromide with metallic sodium (Fittig's reaction):



(2) By heating benzene with methyl iodide in presence of anhydrous aluminium chloride (Friedel—Craft reaction):



(3) By heating the sodium salt of toluic acid with soda lime:



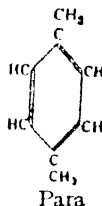
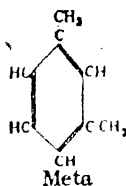
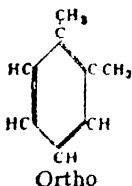
Preparation.—On a large scale, toluene is obtained by a careful fractional distillation of the 2nd fraction of *light oil* (see p. 268) distillate (B.P. 70° to 140°).

Properties, Reactions and Uses.—Toluene is a colourless liquid with a characteristic odour. It has a sp. gr. of 0.865 at 20° . It boils at 110.8° and solidifies at -102° . It is almost insoluble in water but dissolves in the usual organic solvents. On oxidation, the side chain (CH_3 group) is oxidized to COOH forming benzoic acid: $\text{C}_6\text{H}_5\cdot\text{CH}_3 \rightarrow \text{C}_6\text{H}_5\cdot\text{COOH}$.

Toluene is used as a solvent and also as a starting material for some organic compounds used in medicine. It is also used as a preservative in biochemical experiments.

Xylenes, Dimethyl benzenes, $\text{C}_6\text{H}_4(\text{CH}_3)_2$.

The xylenes exist in the following three isomeric forms. They all occur in coal tar from which they are obtained by fractional distillation, the meta-compound being predominant in coal tar.



Properties.—The xylenes are colourless inflammable liquids with a peculiar odour. On oxidation, they yield in the first place monocarboxylic acids, the three (o-, m- and p-) *toluic acids* $\text{C}_6\text{H}_4(\text{CH}_3)\text{COOH}$. On further oxidation of the second methyl group is attacked with the production of dibasic acids, known as (o-, m- and p-) *phthalic acids* $\text{C}_6\text{H}_4(\text{COOH})_2$. *Commercial xylene* or *xylol* is generally a mixture of the three isomers. It is used as a hardening and clearing reagent for histological sections.

o-Xylene; b.p. 142° , sp. gr. 0.8633 at 20° .
m-Xylene; b.p. 139° , sp. gr. 0.8642 at 20° .
p-Xylene; b.p. 138° , sp. gr. 0.8611 at 20° .

Mesitylene, 1:3:5 or *Symmetrical trimethyl benzene*,
 $C_6H_3(CH_3)_3$:

This is a colourless liquid which occurs in coal tar and in some petroleum oils. As stated earlier (see acetone), it can be produced by the condensation of acetone with concentrated sulphuric acid. It boils at 164° and has a sp. gr. of 0.856 at 20° .

CHAPTER XXIII

HALOGEN, NITRO, AMINO, DIAZONIUM, DIAZO AND AZO COMPOUNDS

Halogen Derivatives

The halogen derivatives of benzene and its homologues may be of the following three types:

I. Additive Compounds, such as benzene hexachloride $C_6H_6Cl_6$, benzene hexabromide $C_6H_6Br_6$, etc.

II. Substitution Compounds, with halogens in the *benzene nucleus*, such as monochlorobenzene C_6H_5Cl , dichlorobenzene $C_6H_4Cl_2$, monochlorotoluene $C_6H_4(CH_3)Cl$, etc. These compounds are very stable, and the halogen atom attached to the benzene nucleus is not easily replaced by OH or other groups. They may be prepared either by the action of dry chlorine on benzene in the presence of halogen carriers such as iodine, ferric chloride, etc., or by the Sandmeyer's reaction on a diazonium salt prepared from an aromatic amine (see later).

Monochlorobenzene, Phenyl chloride, C_6H_5Cl : This is prepared (1) either by the action of dry chlorine upon benzene in presence of aluminium mercury couple, $C_6H_6 + Cl_2 = C_6H_5Cl + HCl$, or (2) by warming benzene diazonium chloride (see) with a solution of cuprous chloride dissolved in HCl (Sandmeyer's reaction), $C_6H_5N_2Cl = C_6H_5Cl + N_2$. It is a colourless mobile liquid insoluble in water, b.p. 132° .

p-Dichlorobenzene, $C_6H_4Cl_2$

Prepared by the action of chlorine on benzene in presence of a catalyst like anhydrous aluminium chloride; colourless plates, m.p. 53° , b.p. 173° ; sublimes at room temperature. It is a powerful *insecticide* and used as a substitute for naphthalene.

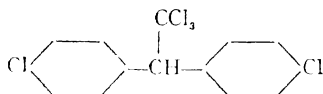
o-Chlorotoluene, $C_6H_4(CH_3)Cl$: prepared either by chlorinating toluene in presence of a halogen carrier, or better by the Sandmeyer's reaction from the diazonium chloride obtained from o-aminotoluene; it is a colourless liquid, b.p. 156° .

Insecticides, Natural and Synthetic

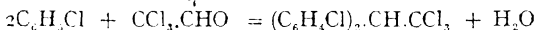
Natural.—Of the various plants which contain substances having strong insecticidal action, the two most important are (1) *Pyrethrum cinerariaefolium* Trev., (Family Compositae), whose flower heads contain the active principles Pyrethrin I and Pyrethrin II, and (2) *Derris elliptica* Benth. and other plants (Lonchocarpus, Tephrosia, etc.) of the family Leguminosae, whose roots contain the active principles rotenone, toxicarol, deguelin, and other allied compounds.

Synthetic.—Of the synthetic compounds the most important are D.D.T. and Gammexane.

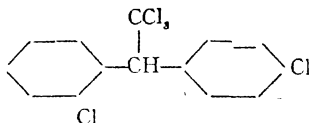
D.D.T., *Dichloro-diphenyl-trichloroethane*, 1-Trichloro-2,2-bis (*p*-chlorophenyl) ethane, *p,p'*-D.D.T.



This can be prepared in the laboratory by the action of conc. H_2SO_4 on a mixture of dry chloral and monochlorobenzene:

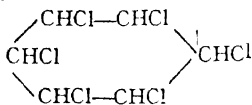


When crystallized from hot alcohol, *pure D.D.T.* is obtained as a white waxy solid, m.p. $108.5^\circ\text{--}109.0^\circ$ (corr.). It is insoluble in water, soluble in hot alcohol, in ethyl acetate, chloroform, benzene, kerosene, and many other organic solvents. Its solution in kerosene (about 5 per cent) is used as a spray for insecticidal action. *Technical D.D.T.* consists of about 70 per cent or more of the pure D.D.T. (*p,p'*-D.D.T.) with other inactive components the major impurity being 1-trichloro-2-o-chlorophenyl -2-*p*-chlorophenyl ethane (o, *p'*-D.D.T.):—



Purified or *aerosol D.D.T.* is a partially refined grade containing greater amounts of *p, p'* D.D.T. (the active component) and having a melting point not less than 103° .

Gammexane, *Hexachlorocyclohexane*, 666, *Benzene hexachloride*, $\text{C}_6\text{H}_6\text{Cl}_6$,



This is the gamma (γ) isomer of benzene hexachloride and is the most toxic of all the four isomers (α , β , γ & δ) hitherto known. It is prepared by the chlorination of benzene and the pure γ -form is

separated from the other isomers by the use of methyl alcohol and chloroform. The pure compound is a colourless substance, m.p. 112° - 113° . It is insoluble in water but soluble in organic solvents such as methyl alcohol, chloroform, carbon tetrachloride, xylene, kerosene, etc. It is a good larvicide and is effective against most insects. It is used either by dilution with an inert solid like gypsum or as its solution in some organic solvent, usually kerosene.

III. Substitution Compounds with halogen atoms in the side chain, e.g., benzyl chloride, benzal chloride, etc. These compounds are generally reactive and resemble the aliphatic derivatives in their chemical behaviour.

Benzyl chloride, $C_6H_5.CH_2Cl$.—This is prepared by passing chlorine into a mixture of boiling toluene and phosphorus pentachloride in presence of sunlight until the required amount of chlorine has been absorbed, and is purified by fractional distillation. It is a colourless liquid with an irritating smell, b.p. 176° , sp. gr. 1.100 at 20° . It is really the halide ester of benzyl alcohol $C_6H_5.CH_2OH$ and on treating this with an alkali it is hydrolyzed to this alcohol.

Benzal chloride, Benzylidene dichloride, $C_6H_5.CHCl_2$.—This is prepared in the same manner as benzyl chloride, only the reaction is allowed to proceed further. It is a colourless liquid, b.p. 207° , sp. gr. 1.256 at 14° . It is used in the preparation of benzaldehyde (see).

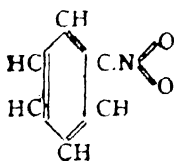
Benzo trichloride, Phenyl chloroform, $C_6H_5.CCl_3$.—This is prepared like the above compound. It is a colourless liquid, b.p. 213° , sp. gr. 1.380 at 14° . It is converted by milk of lime to benzoic acid (see).

Nitro Compounds

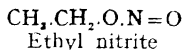
Benzene and its derivatives are easily acted on by concentrated nitric acid, forming nitro compounds. According to the concentration of the acid, the temperature, and the nature of the compounds acted on, one or more nitro groups enter the molecule by displacing the hydrogen atoms in the nucleus.

The nitration is usually carried out by a mixture of concentrated nitric and sulphuric acids. The sulphuric acid takes up the water formed in the reaction and the action of nitric acid becomes more steady and powerful. In these nitro compounds, the nitrogen is directly linked with the carbon atom, whereas in the esters of nitrous acid, which also contain the nitro group, the nitrogen is linked to

oxygen. Thus, nitrobenzene and ethyl nitrite have the following structural formulæ:



Nitrobenzene

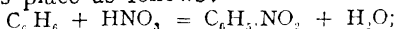


General Properties of Nitro Compounds.—

Usually they are pale yellow substances, volatile in steam, insoluble in water but soluble in alcohol, ether, etc. On reduction in acid solution, they yield amines which are the starting materials for the preparation of various dyes. On heating, the nitro compounds are likely to explode, some of them being used as explosives (see below). The importance of nitro compounds is due to these two considerations.

Nitrobenzene, $\text{C}_6\text{H}_5\text{NO}_2$.

Preparation.—The quantity of benzene to be nitrated is weighed and taken in a large flask. An amount of conc. (sp. gr. 1.4) nitric acid (about $1\frac{1}{2}$ times the theoretical amount) is mixed with the same volume of conc. H_2SO_4 in a separate vessel and the mixture cooled. The mixture of acids is added in small amounts to the benzene which is continuously shaken and cooled under a tap so that the temperature does not exceed 50° , a higher temperature favouring the formation of dinitrobenzene. When the whole of the mixture has been added, the reaction is completed by keeping it over a water bath between 50° — 60° . The mixture is cooled and the nitrobenzene, which floats as an oil, is separated with the help of a separating funnel. It is washed with water and then with dilute sodium carbonate solution, dried over fused CaCl_2 and filtered. The dried liquid is distilled and the fraction between 204° and 207° is collected separately. The reaction takes place as follows:



the concentrated sulphuric acid acts as a dehydrating agent.

Properties and Uses.—Nitrobenzene is a pale yellow liquid with an odour of bitter almonds, b.p. 206° . It has a

sp. gr. of 1.205 at 20°. It is soluble in alcohol and other organic solvents. It is used under the name of *Oil of Mirbane* as a cheap scent for soaps but mainly for the preparation of aniline. It is a poisonous substance and cases of fatal poisoning by nitrobenzene are on record.

m-Dinitrobenzene, $C_6H_4(NO_2)_2$.—This can be prepared from nitrobenzene by the action of a mixture of strong nitric and sulphuric acids and heating on a sand bath. On completion of the reaction and cooling, the m-dinitro compound separates out as a hard yellow cake, crystalline in nature, almost insoluble in water and volatile in steam. It is purified by recrystallization from alcohol, and forms almost colourless plates or needles, m.p. 90°. It is sometimes used as an explosive (D.N.B.).

Nitration of Toluene

Mononitrotoluenes, $C_6H_4(CH_3)NO_2$.—If toluene is nitrated in the same way as described in the case of benzene, the products obtained are *ortho*- and *para*-nitrotoluenes in almost equal quantities. The para compound is solid, m.p. 54°, and the ortho compound is liquid, b.p. 223°, at ordinary temperature. They can, therefore, be separated by freezing. The *meta*-compound, m.p. 16°, cannot be obtained by the above process of nitration of toluene but must be prepared by an indirect method which consists in nitrating a derivative of toluene in which the para position is already occupied.

Dinitrotoluenes, $C_6H_3(CH_3)(NO_2)_2$.—The nitration of the mononitrotoluenes yields the dinitro compounds in which the nitro groups usually occupy the positions 2:4 to the methyl group (m.p. 70°).

Trinitrotoluene, $C_6H_2(CH_3)(NO_2)_3$.—The nitro compound having the nitro groups in the positions 2:4:6 to the methyl group, obtained by the nitration of toluene, is known as *T.N.T.* and is a powerful explosive; m.p. 81°. *Amatol* is the technical name given to T.N.T. mixed with a known proportion of ammonium nitrate, and is a high explosive.

Position of Groups entering the Nucleus

Certain empirical rules may be framed from a study of the known products regarding the relative positions taken up by Cl, Br, NO_2 , and SO_3H groups while entering the nucleus.

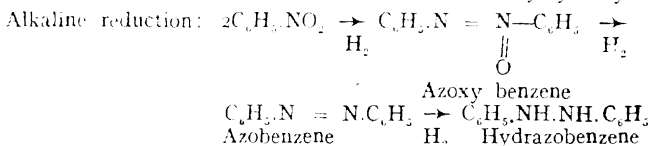
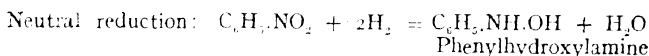
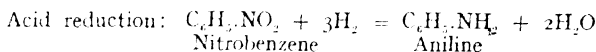
In presence of the groups Cl, Br, I, NH_2 , OH or CH_3 already in the ring, para-compounds with varying amounts of ortho-compounds are formed.

Meta-compounds are the main products when one of the following groups are already present:



Reduction of Nitro Compounds

When reduced in acid solution (Zn dust + acid) nitro compounds yield amino compounds. In neutral solution (Zn dust or aluminium-mercury couple and water) the product is a hydroxylamine. In alkaline solution (Na-methylate, Zn dust and NaOH, or SnCl_2 and NaOH), azoxy-, azo- and hydrazo-compounds in successive steps are formed. The following equations will illustrate the above statements:



At the *cathode* of an electric cell, different *reduction products* have been obtained by varying the conditions (electrolytic reduction).

Aromatic Amino Compounds or Arylamines

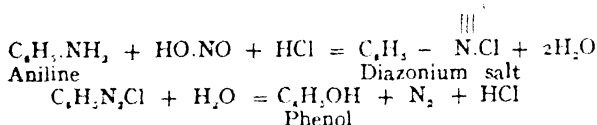
These compounds are derived from benzene and its derivatives by the substitution of the hydrogen atoms of the nucleus by amino groups. According to the number of hydrogen atoms substituted, we have, mono-, di-, and tri-amino compounds. It may be noted that the term *amino compound* or *aryl-amine* is used instead of the term *amine* as there are certain differences between the behaviour of these compounds and the aliphatic amines. Like the aliphatic amines, these are divided into three classes: (1) *primary amines*, e.g., aniline $\text{C}_6\text{H}_5\text{NH}_2$, (2) *secondary amines*, e.g., diphenylamine $(\text{C}_6\text{H}_5)_2\text{NH}$, methylaniline $\text{C}_6\text{H}_5\text{NH.CH}_3$ (mixed amine), etc., and (3) *tertiary amines*, e.g., triphenylamine $(\text{C}_6\text{H}_5)_3\text{N}$, dimethylaniline $\text{C}_6\text{H}_5\text{N}(\text{CH}_3)_2$, etc.

Preparation and Properties of Aromatic Amino Compounds.—The preparation of a primary amine is carried out by the reduction of a nitro compound in acid solution by means of tin, zinc or iron with acetic or hydrochloric acid.

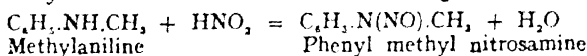
When prepared fresh, they are generally colourless liquids or solids with a peculiar odour and can be distilled unchanged. The secondary and tertiary amines are prepared by heating under pressure in an autoclave a mixture of a primary amine and a halogen derivative of the hydrocarbon.

The primary amines are very little soluble in water but dissolve easily in alcohol, ether, etc. The primary amines are weak bases and form water-soluble salts with mineral acids but the secondary amines and tertiary amines are too weak bases to form stable water-soluble salts.

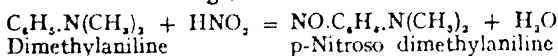
The three types of amines differ in their action on *nitrous acid*. A *primary amine* when treated with nitrous acid in the cold gives a diazonium salt soluble in water. If, however, the solution is warmed the diazonium salt breaks up into a phenol with the evolution of nitrogen: e.g.,



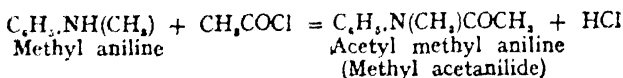
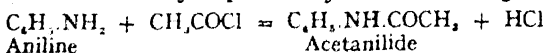
A *secondary amine* with nitrous acid yields a nitroso compound, a yellow oil insoluble in water: e.g.,



A *tertiary amine* with nitrous acid gives a coloured nitroso compound in which the nitroso group (NO) is linked with the benzene nucleus: e.g.,



When treated with *acetyl chloride*, primary and secondary amines yield anilides but the tertiary amine is without any action since the last available hydrogen atom of the amino group has been already replaced by a radical: e.g.,

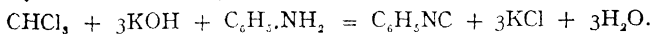


Aniline, Aminobenzene, Phenylamine, $C_6H_5.NH_2$.

Preparation.—Aniline is prepared in the laboratory by reducing nitrobenzene with tin and hydrochloric acid: $C_6H_5.NO_2 + 3H_2 = C_6H_5.NH_2 + 2H_2O$. A weighed quantity of nitrobenzene is taken in a round bottom flask, fitted with an air condenser, and about twice the weight of granulated tin is added, and the liquid is warmed on the water bath for a few minutes. Concentrated HCl (about 4 times the bulk of the nitrobenzene taken) is now added in 10 c.c. lots to the above mixture, which is cooled between the additions. When all the acid has been added, the flask is heated on the water bath for about an hour to complete the reduction. Aniline is then liberated from the double salt formed with stannic chloride, $(C_6H_5.NH_2.HCl)_2.SnCl_4$, by the addition of a solution of NaOH until the reaction is strongly alkaline, and it is next distilled off in a current of steam. The distillate is extracted with ether, the ethereal solution dehydrated with solid KOH, the ether recovered by distillation and the residual aniline purified by distillation.

Properties, Reactions and Uses.—Aniline is a poisonous, oily liquid with a faint peculiar odour. When distilled, it has a pale yellow colour but turns brown when exposed to light and air. It has a sp. gr. of 1.022 at 20°. It is sparingly soluble in water but is miscible with organic solvents such as alcohol, ether, etc. It boils at 184° and is volatile in steam. It burns with a smoky flame. It is a *weak base* and forms soluble salts with acids, e.g., aniline hydrochloride $C_6H_5.NH_2.HCl$, aniline sulphate $(C_6H_5NH_2)_2.H_2SO_4$, etc.

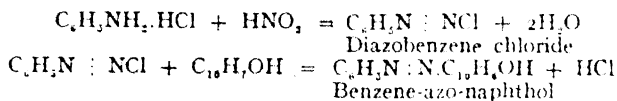
When aniline is heated with chloroform and alcoholic potash, phenyl isocyanide or phenylcarbylamine C_6H_5NC is formed which is readily detected by its very offensive odour (*carbylamine reaction*):



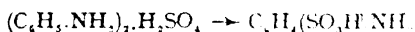
A solution of bleaching powder or of sodium hypochlorite gives with aniline an intense violet colour which turns brown and then fades. When bromine-water is added to aniline hydrochloride, there is a precipitate of symmetrical tri-bromaniline, $C_6H_2Br_3NH_2$.

Diazo-reaction: A few drops (8 or 10) of a dilute solution of sodium nitrite are added to a well-cooled solution

of aniline in dilute HCl and then a few drops of a solution of α - or β -naphthol dissolved in NaOH are added; there is a bright scarlet colour due to the formation of benzene-azonaphthol:



When aniline sulphate is heated at 180° - 200° , *sulphanilic acid* is formed, the sulphonic acid group occupying the para position:

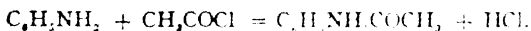


Conc. nitric acid usually decomposes aniline. If, however, aniline is treated with nitric acid in presence of conc. sulphuric acid at a low temperature, *meta-nitraniline* is formed. The ortho- and para-nitranilines are not obtained by direct nitration but can be produced by the nitration of acetanilide.

Aniline is used in the preparation of various dyes, such as magenta, mauveine, safranine, etc., and it is also the starting material for many synthetic drugs, the sulphanilamide group being the most important one (see).

Acetanilide, *Antifebrin*, $\text{C}_6\text{H}_5\text{NHCOCH}_3$.

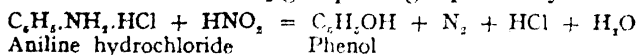
This is prepared by boiling aniline with a mixture of glacial acetic acid and acetyl chloride under a reflux condenser. The mass is then poured into water and the precipitated acetanilide recrystallized from hot water.



Acetanilide is a white crystalline solid used in medicine as an antipyretic, whence the name antifebrin. It melts at 114° .

Diazonium Salts and Diazo Compounds

When a primary aliphatic amine is acted on by nitrous acid, nitrogen is evolved and the corresponding alcohol is produced (see). When a primary aromatic amine is acted on by nitrous acid and warmed, nitrogen is evolved and a phenol is formed, the NH_2 group being replaced by OH , e.g.,

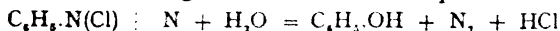


If however, the aryl amine is acted on by nitrous acid in a well-cooled solution, an intermediate compound, known as

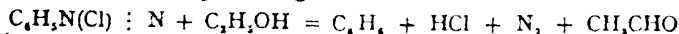
The *diazonium* compounds are ionizable and differ from *diazo* compounds which contain the group —N:N— and are not ionizable. Examples of diazo compounds are, diazoaminobenzene $\text{C}_6\text{H}_5\text{.N:N.NH.C}_6\text{H}_5$, diazoaminotoluene $\text{C}_6\text{H}_5\text{.N:N.NH.C}_6\text{H}_4\text{.CH}_3$, etc.

Reactions of Diazonium Salts.—The diazo reaction has been very useful for the preparation of various aromatic compounds, specially in the synthesis of azo dyes. A few reactions of theoretical interest are mentioned below and since the diazonium salt is prepared from a primary amine, each reaction shows the replacement of an amino group by the corresponding radical:

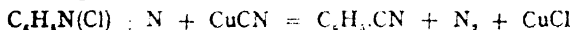
1. *Replacement of a diazonium group by a hydroxyl group.*—If an aqueous solution of a diazonium compound is warmed, nitrogen is evolved and a phenol is formed, *e.g.*,



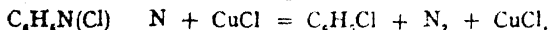
2. *Replacement of the diazonium group by hydrogen.*—If a diazobenzene salt is boiled with absolute alcohol, the diazo group is replaced by hydrogen and the alcohol is oxidized to aldehyde: *e.g.*,



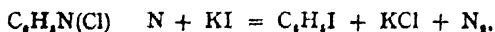
3. *Replacement of the diazonium group by a cyanogen group.*—This is done by adding diazobenzene chloride solution to a solution of cuprous cyanide in potassium cyanide and warming (*Sandmeyer's Reaction*): *e.g.*,



4. *Replacement of the diazonium group by halogens.*—When a diazonium compound is added to a concentrated solution of cuprous chloride in HCl and warmed, nitrogen is evolved and the diazo group is replaced by chlorine: *e.g.*,

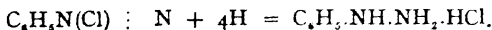


Warming with cuprous bromide in hydrobromic acid gives a bromo derivative and warming of the diazonium compound with potassium iodide gives the iodo compound (*Sandmeyer's Reaction*):



Phenylhydrazine, $C_6H_5.NH.NH_2$

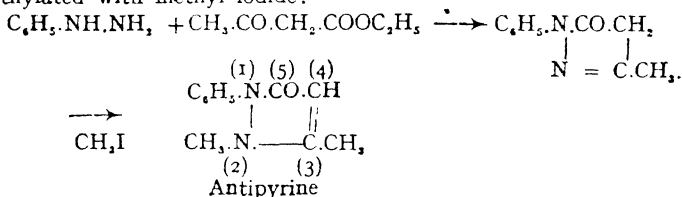
This important compound is prepared by reducing benzene diazonium chloride with stannous chloride in HCl:



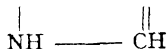
The phenylhydrazine hydrochloride is filtered off, the base liberated with NaOH and extracted with ether. The ether is removed by evaporation and the phenylhydrazine purified by distillation under reduced pressure.

When freshly distilled, phenylhydrazine is a colourless liquid, b.p. 243° . On keeping it gradually turns brown. It is only sparingly soluble in water but dissolves easily in alcohol and ether. It is a strong base and forms salts with acids which dissolve in water and crystallize well. It reacts readily with aldehydes and ketones giving phenylhydrazones. It reacts with sugars giving hydrazones and osazones (see). Many substituted phenylhydrazines are also used in the identification of sugars. Besides the use of phenylhydrazine as a reagent it largely used in the preparation of antipyrine.

Antipyrine, 1-Phenyl-2:3-dimethyl-5-pyrazolone.—This is a heterocyclic compound prepared by the condensation of phenylhydrazine with ethylacetoacetate, and the product so obtained is methylated with methyl iodide:



It is thus a derivative of *pyrazole*, a heterocyclic compound having the structure $N = CH - CH$. Antipyrine is a colourless crystalline

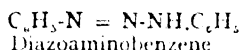
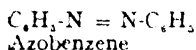


substance, soluble in water and alcohol, m.p. 113° . It is a monacid base and forms salts, most of which are soluble in water. Its aqueous solution gives a red colour with ferric chloride and a green colour with nitrous acid. It has a marked analgesic action and is a very useful antipyretic. *Salipyrine* (antipyrine salicylate) and *pyramidone* (4-dimethylamino antipyrine) are some of its derivatives used in medicine.

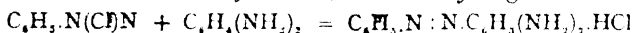
AZO COMPOUNDS

The group $-N=N-$ is characteristic of both *diazo* compounds mentioned above as well as for *azo* compounds. In

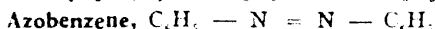
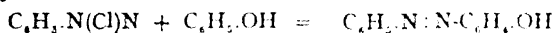
azo compounds, each of the doubly linked nitrogen atoms is attached to a benzene nucleus and hence these azo compounds are more *stable* than the diazo compounds in which at least one of the doubly linked nitrogen atoms is not so attached: *e.g.*,



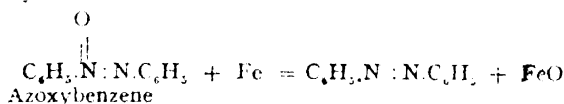
The diazonium salts react with aromatic amines, phenols, etc., and produce azo compounds some of which have proved to be valuable dyes. This process is generally known as *coupling*: *e.g.*, benzene diazonium chloride coupled with *m*-phenylene diamine gives diaminoazobenzene hydrochloride, known as *chrysoidine*, used in dyeing wool and silk:



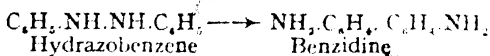
Again, benzene diazonium chloride coupled with phenol gives hydroxyazobenzene:



This may be prepared by the reduction of nitrobenzene by zinc dust and NaOH: $2\text{C}_6\text{H}_5\text{.NO}_2 + 4\text{H}_2 = \text{C}_6\text{H}_5 - \text{N:N} - \text{C}_6\text{H}_5 + 4\text{H}_2\text{O}$; or, by the action of iron filings upon azoxybenzene, the latter being obtained by the reduction of nitrobenzene with sodium methylate:



Azobenzene is reduced to hydrazobenzene $\text{C}_6\text{H}_5\text{.NH.NH.C}_6\text{H}_5$ by zinc dust and NaOH. It breaks up into aniline when reduced with SnCl_2 and HCl. Hydrazobenzene undergoes a curious intramolecular change when it is treated first in the cold and then warmed with HCl:



This process is known as the *benzidine conversion*. Benzidine is a valuable substance for the preparation of certain dyes.

Azo Dyes

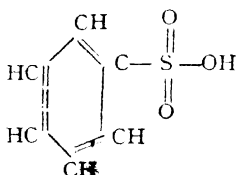
The azo dyes are prepared from a diazotized amine coupled with amines, phenols, etc. Apart from their use as dyes, some are used as *indicators*. Examples of azo dyes and indicators are chrysoidine, methyl orange, Biebrich scarlet, Congo red, Sudan III, etc.

CHAPTER XXIV

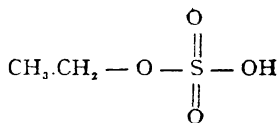
SULPHONIC ACIDS AND PHENOLS

Aromatic Sulphonic Acids

A characteristic property of the aromatic hydrocarbons is to form sulphonic acids when acted upon by concentrated sulphuric acid. These acids are derivatives of sulphuric acid, the OH group of which is replaced by an aryl radical. The carbon atom of a benzene ring is in this case directly linked with the sulphur atom whereas in the case of the sulphuric acid esters the carbon atom is linked with sulphur through an atom of oxygen:

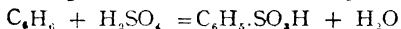


Benzene sulphonic acid



Ethyl hydrogen sulphate

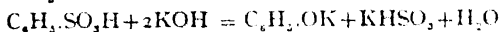
The monovalent group $\text{—SO}_2\text{.OH}$ or SO_3H , known as the *sulphonic acid group*, is introduced into the nucleus by agitating an aromatic hydrocarbon with concentrated sulphuric acid, and the process is known as *sulphonation*:



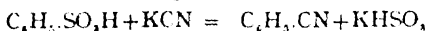
When the sulphonation of a hydrocarbon like benzene is carried out more energetically with fuming sulphuric acid, we may get ortho-, meta- and para-*disulphonic acids* or even *trisulphonic acids*.

The sulphonic acids are usually soluble in water and have a strong acid reaction, the hydrogen atom of the sulphonic acid group being replaceable by metals forming salts. Their basicity depends upon the number of SO_3H groups present. When heated to a high temperature, they decompose and they do not show any sharp melting points.

When fused with caustic potash, they yield phenols in the form of alkali phenates from which the free phenols can be obtained by treatment with a mineral acid: *e.g.*,



It is important to note, therefore, that phenols can be prepared from benzene through the sulphonic acids as well as through the nitro compounds. When distilled with potassium cyanide the sulphonic acids yield nitriles; *e.g.*,



For other reactions of sulphonic acids, see benzene sulphonic acid.

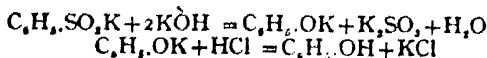
Benzene sulphonic Acid, $\text{C}_6\text{H}_5\text{SO}_3\text{H}$

Preparation.—This is easily prepared in the laboratory by gradually adding benzene (30g.) in small quantities to fuming sulphuric acid (120 g.) kept in a round bottomed flask fitted with a reflux condenser. The flask is cooled after each addition and after the reaction is complete the mass is poured into an excess of water (500 c.c.). The solution is neutralized with powdered BaCO_3 , warmed and filtered hot. The filtrate is concentrated and cooled when the barium salt-crystallizes out. It is purified by recrystallization from hot water. The barium salt is treated with the calculated quantity of dilute sulphuric acid and the BaSO_4 filtered off. The sulphonic acid is obtained by evaporating the solution.

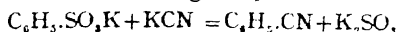
Properties and Reactions.—Benzene sulphonic acid is a colourless hygroscopic solid, m.p. 50° . Its aqueous solution is strongly acid in reaction and forms salts with metals, the barium, calcium and lead salts being soluble in water. On heating it is decomposed. On heating with conc. HCl at 150° it is decomposed into benzene and sulphuric acid:



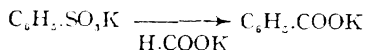
When fused with an alkali it gives phenol as its alkali salt from which the free phenol can be regenerated with a mineral acid:



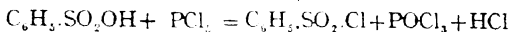
When distilled with KCN it gives benzonitrile:



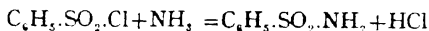
Benzonitrile on hydrolysis gives benzoic acid, and the method, therefore, enables the introduction of COOH group into the benzene nucleus through the sulphonic acid: $\text{C}_6\text{H}_5\cdot\text{CN} \rightarrow \text{C}_6\text{H}_5\cdot\text{COOH}$. The COOH group can also be introduced directly by fusing the potassium salt of sulphonic acid with potassium formate when potassium benzoate is formed:



When heated with phosphorus pentachloride, it gives *benzene sulphonic chloride*:



When benzene sulphonic chloride is treated with ammonia, it gives *benzene sulphonamide*:

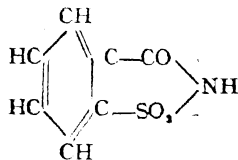


Benzene sulphonic chloride also reacts with primary and secondary amines giving sulphonamides, and it is therefore used for differentiating between the three types of amines (see aliphatic amines).

Toluene sulphonic Acids

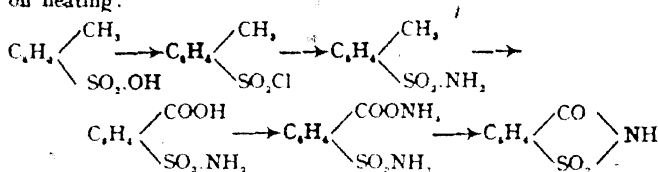
The ortho- and para-toluene sulphonic acids are obtained by the direct sulphonation of toluene, and these are the starting materials for the two important compounds described below.

Saccharin, o-Sulphobenzoimide,

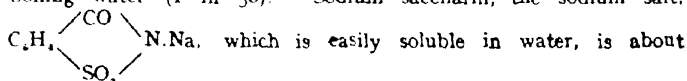


Preparation.—The mixture of o- and p-toluene sulphonic acids obtained by the sulphonation of toluene is treated with phosphorus pentachloride which gives a mixture of o- and p-toluene sulphonic chlorides. The ortho compound which is a liquid is then separated from the para compound which is

solid. The ortho-toluene sulphonic chloride is treated with ammonia under pressure when o-toluene sulphonamide is formed. The amide is oxidized with alkaline potassium permanganate to o-sulphamido-benzoic acid, and the ammonium salt of this acid gives saccharin on heating:

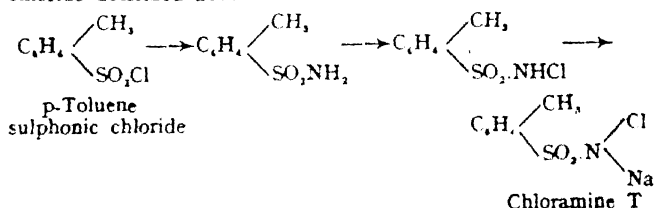


Properties.—Saccharin is a colourless crystalline solid, m.p. 227° . It is about 500 times sweeter than cane sugar and is used as a sweetening agent for diabetics and obese persons. It is sparingly soluble in cold water (1 in 232) but fairly soluble in boiling water (1 in 30). Sodium saccharin, the sodium salt,

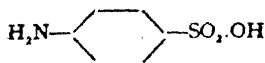


400 times sweeter than cane sugar. The ammonium salt, known as Sucramine, is about 700 times sweeter than cane sugar.

Chloramine T.—This is a powerful antiseptic prepared by the action of sodium hypochlorite upon p-toluene sulphonamide obtained by the action of ammonia upon p-toluene sulphonic chloride described above:



Sulphanilic Acid, p-Aminobenzene sulphonic Acid,



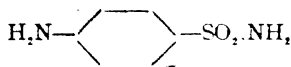
Preparation.—Concentrated sulphuric acid (80 g.) is taken in a round bottom flask, and aniline (25 g.) is gradually added. The flask is then heated over an oil bath at 180° – 190° for 4 to 5 hours and the product poured gradually into an excess of water. The precipitated sulphanilic acid is filtered off and recrystallized from hot water.

Properties, Reactions and Uses.—This is a colourless crystalline substance which has no definite melting point. It is sparingly soluble in cold water but more so in hot. It is used as a reagent but mainly as the starting material for various dyes and drugs.

Sulphanilamide or Sulphonamide Group of Drugs

As in the case of benzene sulphonic acid, the OH group of the sulphonic acid radical in sulphanilic acid can be replaced by an amino group giving sulphanilamide (see below). Sulphanilamide and its numerous derivatives containing a sulphur atom in the para position to the amino group have proved to be valuable internal antiseptics and are used in the treatment of diseases produced by infection with streptococcus, staphylococcus, pneumococcus, gonococcus, etc. This discovery of the treatment of bacterial diseases by synthetic chemotherapeutic compounds have opened out immense possibilities, and has earned the Nobel Prize for Domagk in 1939. Innumerable substituted sulphonamides have been synthesized, and the following are only a few of the important compounds used in medicine. For tests and other informations see Chapter 35.

1. **Sulphanilamide**, *p*-Aminobenzene sulphonamide, *Prontosil album*, *Streptocide*, etc.

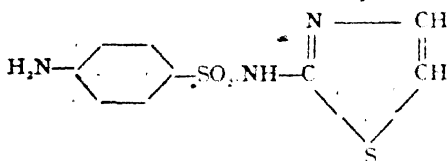


This is prepared by the hydrolysis of the amide of acetyl sulphanilic acid with HCl; a white crystalline powder, m.p. 164.5° to 166.5°, having a slightly bitter with a sweet after-taste; insoluble in ether, chloroform or benzene. One gram dissolves in 115 c.c. water, in 37 c.c. alcohol, and in 5 c.c. acetone at 25°; easily soluble in boiling water, soluble in glycerol, in aqueous HCl (1 in 10 of dil.) and in aqueous NaOH (1 in 5 of 10 per cent). According to B.P., it can be estimated by dissolving it in dilute HCl and titrating at a temperature below 15° with M/10 solution of sodium nitrite, using starch-iodide paste as indicator. Each c.c. of M/10 sodium nitrite is equivalent to 0.01722 g of $\text{C}_6\text{H}_5\text{O}_2\text{N}_2\text{S}$. It is active against haemolytic streptococci, but relatively feebly active against pneumococci and meningococci.

1(a). **Sulphacetamide**, *p*-Aminobenzene sulphonacetamide, $H_2N.C_6H_4.SO_2.NH.CO.CH_3$: Prepared by the acetylation of sulphanilamide with acetic anhydride. It is a white or yellowish white crystalline powder with a slightly saline and an acid taste; m.p. 181° — 184° ; soluble in 150 parts of water and in 15 parts of alcohol at 20° ; insoluble in ether but soluble in 7 parts of acetone; soluble in mineral acids and in solutions of alkali carbonates; used in eye infections; assayed as in sulphanilamide, each c.c. of M/10 $NaNO_2$ being equivalent to 0.02142 g of $C_8H_9O_3N_2S$.

Sulphacetamide Sodium or **Soluble Sulphacetamide**, is a white or yellowish white micro crystalline powder with a slightly bitter taste; soluble in 1.5 parts of water at $15.5^\circ C$, sparingly soluble in alcohol and in acetone.

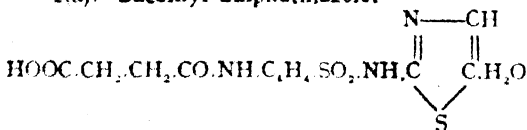
Sulphathiazole, 2-(*p*-Aminobenzene sulphonamidol-thiazole



This is prepared by the action of *p*-acetamidobenzene sulphonyl chloride upon 2-aminothiazole and subsequent hydrolysis of the acetyl group. It is a white or yellowish white tasteless powder, m.p. 200° — 203° ; it is only slightly soluble in water or alcohol but dissolves easily in dilute mineral acids and in solutions of alkali hydroxides and carbonates. The compound is active against pneumococcus, meningococcus, gonococcus, staphylococcus and plague bacillus. Sulphathiazole is assayed in the same way as sulphanilamide, each c.c. of M/10 $NaNO_2$ being equivalent to 0.02553 g. of $C_8H_8O_3N_2S_2$.

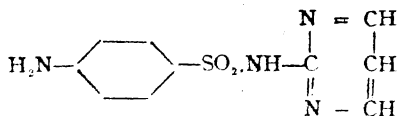
Sulphathiazole Sodium or **Soluble Sulphathiazole**, is the pentahydrate of the sodium derivative of sulphathiazole, the Na atom replacing the H atom of the $-SO_2.NH-$ group. It is soluble in about 3 parts of water and in about 20 parts of 95 per cent alcohol at $15.5^\circ C$.

2(a). **Succinyl Sulphathiazole**.



Prepared by condensing sulphathiazole with succinic acid; a white or yellowish white crystalline powder, m.p. 188° — 195° ; very sparingly soluble in water, alcohol and acetone; soluble in dil. caustic alkalies and in solutions of sodium bicarbonate with evolution of CO_2 ; used in bacillary dysentery and cholera.

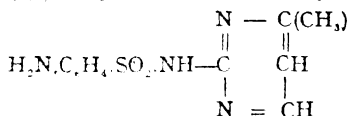
3. **Sulphadiazine**, *Sulphapyrimidine*, 2-(p-Aminobenzene sulphonamido)-pyrimidine



This is prepared by the action of p-acetamidobenzene sulphonyl chloride upon 2-aminopyrimidine and subsequent hydrolysis of the acetyl group with dil. NaOH. It is a white or yellowish white tasteless powder, m.p. $252^\circ-256^\circ$; scarcely soluble in water at 25° , sparingly soluble in alcohol and in acetone; soluble in dilute mineral acids and in aqueous solutions of alkali hydroxides. The action is similar to that of sulphathiazole but is less toxic. It is assayed in the same manner as sulphanilamide, each c.c. of M/10 NaNO_2 being equivalent to 0.02503 g of $\text{C}_{10}\text{H}_{10}\text{O}_2\text{N}_4\text{S}$.

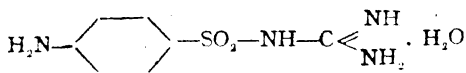
Sulphadiazine Sodium or *Soluble Sulphadiazine* is the sodium derivative of sulphadiazine in which the H atom of $-\text{SO}_2\text{NH}-$ is replaced by Na. It is soluble in about 2 parts of water at 25° and is sparingly soluble in 95 per cent alcohol.

3(a) **Sulphamerazine** or 2-Sulphanilamido-4-methyl pyrimidine.



This monomethyl derivative of sulphadiazine is a white crystalline powder, very slightly soluble in water (1:4500), readily soluble in dil. acids and alkaline hydroxides. It is stated to exert potent bacteriostatic and bactericidal effects on the group of cocci and in certain other organisms including plague bacillus.

4. **Sulphaguanidine**, p-Aminobenzene sulphonyl guanidine monohydrate,



This is prepared by fusing dicyandiamide with p-aminobenzene sulphonamide. It is a colourless, tasteless crystalline powder, which slowly darkens on exposure to light, m.p. $190^\circ-192.5^\circ$ (anhydrous); only slightly soluble in water (1 in 1000 at 25°), soluble in boiling water (1 in 10), sparingly soluble in alcohol and in acetone; easily soluble in dilute mineral acids but insoluble in aqueous solutions of alkali hydroxides. It is active against bacillary dysentery, cholera and infections of the lower bowel. Assayed as in sulphanilamide, each c.c. of M/10 NaNO_2 being equivalent to 0.02142 g. of $\text{C}_7\text{H}_{10}\text{O}_2\text{N}_4\text{S}$.

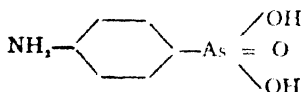
A study of these substituted sulphonamides has shown that of the three types of H atoms present in the sulphonamide molecule, viz., 4 nuclear, 2 amino and 2 sulphonamido H atoms, only the substitution of the *sulphonamido hydrogen atoms* yields a variety of derivatives of medicinal value, as will also be apparent from the structure of some of the active compounds described above.

These drugs are not *bactericidal*, but *bacteriostatic*, that is, they do not kill the bacteria but prevent their multiplication until the natural defence mechanism of the body is able to overcome them. And it has been found that the treatment succeeds best if a high concentration of the drug in the blood stream is produced as quickly as possible with a large initial dose, or the bacteria become sulphonamide resistant.

N.B. A similar group of synthetic drugs, the aromatic *sulphones* (cf. p. 132), have shown bacteriostatic action against tuberculosis and leprosy. Thus, *Promin* or *pp'*-*Diamino-diphenyl sulphone*—*N,N'*-*dextrose sodium sulphonate*, *Diasone* or *Disodium formaldehyde sulphonylate diamino diphenyl sulphone*, and *Promizole* or *4,2'-Diamino phenyl-5'-thiazolyl sulphone*, are stated to have been used in tuberculosis and leprosy with moderate success.

Some Arsenic Compounds Used in Medicine

Arsanilic Acid, *p*-Aminophenylarsonic Acid



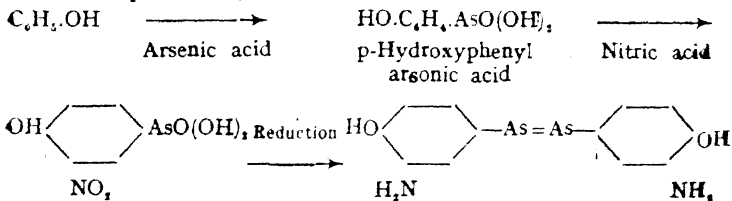
This is prepared by heating aniline with arsenic acid to $190^\circ\text{--}200^\circ$. The monosodium salt is known as *atoxyl*, $\text{NH}_2\text{C}_6\text{H}_4\text{AsO}(\text{OH})(\text{ONa})$. Atoxyl has a marked curative action in cases of trypanosomiasis, syphilis, etc., but unfortunately it is cumulative in action and liable to cause blindness. Its use has been largely replaced by derivatives of trivalent arsenic such as *salvarsan*, etc. The acetyl derivative of arsanilic acid is known as *arsacetin*, $\text{CH}_3\text{CO}\cdot\text{NH}\cdot\text{C}_6\text{H}_4\cdot\text{AsO}(\text{OH})\cdot(\text{ONa})\cdot 5\text{H}_2\text{O}$; this is less toxic than atoxyl, and is prepared by the action of acetic anhydride on arsanilic acid.

Carbarsone, *Amibiarsen*, *4*-Carbamido-phenyl-arsonic Acid
 $\text{NH}_2\cdot\text{CO}\cdot\text{NH}\cdot\text{C}_6\text{H}_4\cdot\text{AsO}(\text{OH})_2$.

This is a white crystalline solid, m.p. 174° . It is stable in air, almost insoluble in water, but soluble in aqueous alkalis. It is used for the treatment of amoebic dysentery and giardiasis. It contains about 29 per cent of arsenic.

Salvarsan, Ehrlich 606, Dihydrochloride of 3:3'-diamino-4:4'-dihydroxy-arsenobenzene.

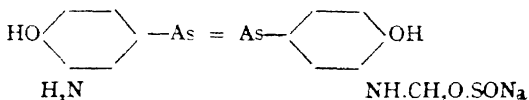
This compound was discovered by Ehrlich and Hata. The different steps in its synthesis are as follows:—



3-Nitro-4-hydroxyphenyl arsonic acid 3:3'-Diamino-4:4'-dihydroxy-arsenobenzene

The free base is a pale yellow powder, soluble in dilute HCl or NaOH. The dihydrochloride is used in the treatment of syphilis and other affections. Its solution is slightly acid in reaction and has to be neutralized before injection. It contains 30 per cent of arsenic although theoretically it should contain 33.6 per cent.

Neosalvarsan, Ehrlich 914, Sodium-3:3'-diamino-4:4'-dihydroxy arsenobenzene-N-methylene sulphonylate.



This is obtained by treating salvarsan with sodium formaldehyde sulphonylate. It is a pale yellow powder, soluble in water giving a neutral solution, and it is owing to this advantage that it has largely superseded salvarsan in clinical use. It contains about 20 per cent of arsenic.

For tests, etc., see Toxicology (pp. 564—566).

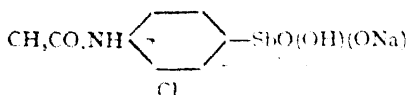
Some Pentavalent Antimony Compounds used in Medicine

It has been mentioned earlier that the trivalent antimony compound sodium antimonyl tartrate has been found to be useful in the treatment of *Kala-azar*. The pentavalent antimony compounds, similar to the corresponding arsenic compounds have, however, proved to be more potent in the treatment of this disease, and several compounds have been synthesized and tried. Only a few of the important compounds are mentioned below:—

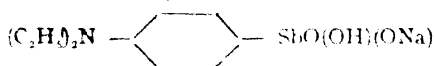
1. **Urea Stibamine** (Brahmachari).

Its active component is probably *s*-Diphenyl carbamido-4:4'-distibonic acid.

2. **Stibosan**, von Heyden 471 *m*-Chloro-*p*-acetyl-amino-phenyl stibonate of sodium.



3. **Neo-stibosan**, von Heyden 693, *p*-Diethylamino phenyl stibonate of sodium.

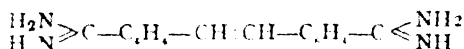


4. **Colustibosan**, *Stibatin*, Sodium antimony gluconate: A pentavalent antimony compound of hexonic acid; white powder, soluble in water; forms a stable solution; toxicity low; used in kala-azar.

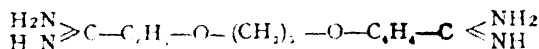
5. **Anthiomaline**, Lithium antimony thiomalate: a 6 per cent solution is used in filariasis, bilharziasis, leishmaniasis, and lymphogranuloma inguinale.

N.B. Besides the above antimony compounds, some aromatic diamidines (cf. amidines, p. 257) have come into use in the treatment of kala-azar and trypanosomiasis; for example,

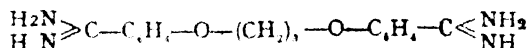
(i) **Stilbamidine** 4:4'-Diamidino stilbene, 4:4'-Diamidino diphenyl ethylene, M. & B. 744.



(ii) **Pentamidine**, 4:4'-Diamidino diphenoxypentane, M. & B. 800.



(iii) **Propamidine**, 4:4'-Diamidino diphenoxypropane, M. & B. 782.



These compounds, used as salts of β -hydroxy ethane sulphonic acid are white powders, readily soluble in water.

Natural Antibiotics

These are organic compounds which are produced by moulds, bacteria, actinomyces, etc. They prevent the multi-

plication of micro-organisms responsible for various types of infection, and have recently come into use in medicine. A large number of these antibiotics have been isolated, e.g., the different Penicillins from *Penicillium notatum*, *P. chrysogenum*, etc., Streptomycin from *Actinomyces griseus*, Clavacin from *Aspergillus clavatus*, Tyrothricin (gramicidin) from *Bacillus brevis*, Streptothricin from *Actinomyces lavendulae*, and so on.

PENICILLINS

At least seven penicillins are now known. They are strong monobasic acids forming crystalline sodium and calcium salts. The following basic structural formula is suggested for them:

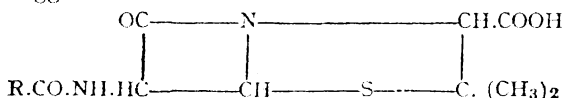


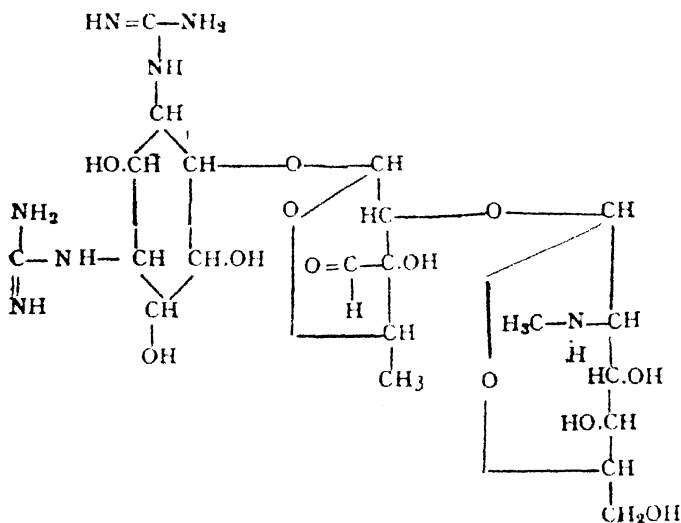
Table showing the names, side chains and activities of the penicillins (Goodall & Levi, Analyst, 1947).

Name		Side Chain (R)	Activity per mg.		Ratio a ÷ b
British	American		against <i>B. subtilis</i> (a)	against <i>S. aureus</i> (b)	
I	F	Δ 2-pentenyl (-CH ₂ .CH:CH.CH ₂ .CH ₃)	970	1550	0.63
II	G	Benzyl (-CH ₂ .C ₆ H ₅)	1667	1667	1.0
III	X	p-hydroxybenzyl (-CH ₂ .C ₆ H ₄ .OH)	800	900	0.89
K	K	n-heptyl (-CH ₂ .(CH ₂) ₅ .CH ₃)	700	2300	0.30
IV, VI & VII }	—	unknown	unknown	unknown	—

Streptomycin

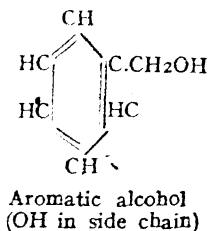
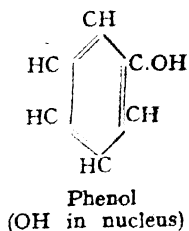
This is obtained from *Actinomyces griseus*; has lately attracted much attention on account of its relatively lower toxicity to animals and greater antibiotic activity particularly against tubercle and plague bacilli.

Streptomycin is an organic nitrogenous base, soluble in water, and has been obtained in crystalline form. Its empirical formula is $C_{21}H_{39}N_7O_{12}$. The following structural formula has been suggested by Kuehl et al (J.A.C.S., 1947):

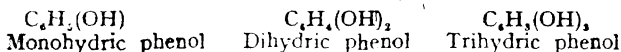


PHENOLS

The name phenol is given to a hydroxy derivative of an aromatic hydrocarbon in which a hydrogen atom of the nucleus (ring) is replaced by OH. The phenols are acidic in nature since the OH group attached to a carbon atom of the ring is *acidic* owing to the negative nature of the phenyl radical. When the OH group enters the side chain and is attached to an alkyl radical, as in aromatic alcohols, it is *alcoholic* in nature. Thus the position of the OH group distinguishes a phenol from an aromatic alcohol, e.g.,



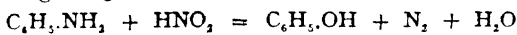
The phenols are classified according to the number of hydroxyl groups which are directly united with the carbon atom of the nucleus. Carbolic acid or monohydroxybenzene may be mentioned as a *monohydric phenol*, resorcinol and its two isomers as *dihydric phenols* and phloroglucinol and its two isomers as *trihydric phenols*. All the six hydrogen atoms of benzene may be replaced by OH groups.



Many of the phenols are found in the products of the destructive distillation of coal, turf, wood, etc., particularly the former. Some complex phenols are found in plants, *e.g.*, thymol in *Ptychotis ajowan* D.C., carvacrol in *Thymus vulgaris* L., eugenol in *Pimenta officinalis* Lindl., and so on. Phenol sulphonic acid is excreted in the urine.

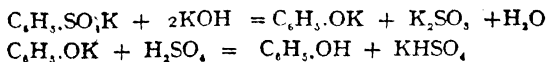
General Methods of Preparation of Phenols

(1) By the action of nitrous acid on an amino compound and warming: *e.g.*,



Phenols can thus be prepared from benzene through the nitro and amino compounds.

(2) By fusing sulphonic acids or their salts with caustic alkalis: *e.g.*,



Phenols can thus be prepared from benzene through the sulphonic acids.

General Properties and Reactions of Phenols

Most of the phenols are soluble in water and alcohol, and usually volatilize in steam; many of them have a strong antiseptic action. They are mostly colourless and crystalline and possess characteristic odours.

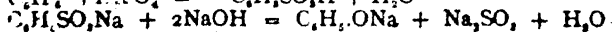
The acidic character of the phenols is shown by the formation of salts with alkali hydroxides. These salts are known as phenates or phenoxides (*cf.*, ethoxide or alcoholate). Carbon dioxide reacts with sodium or potassium phenoxide forming hydroxy acids (*e.g.*, salicylic acid). When heated with zinc dust the phenols are reduced to hydrocarbons:



Like the alcohols, they form ethers such as anisole or methyl-phenyl ether $\text{C}_6\text{H}_5\text{OCH}_3$ and diphenyl ether $\text{C}_6\text{H}_5\text{O.C}_6\text{H}_5$. They also form esters such as phenyl acetate $\text{C}_6\text{H}_5\text{O.CO.CH}_3$. With ferric salts, the phenols usually give a colouration. With mercury nitrate containing nitrous acid a red colour is generally produced. Phenols when warmed with concentrated sulphuric acid and a small crystal of a nitrite added, give blue or green colouration which turns to red on the addition of water and again becomes green on adding NaOH or NH_4OH solution (*Liebermann's reaction*). Phenols react with diazonium salts to form azo dyes.

Phenol, Hydroxybenzene, Carboic Acid. $\text{C}_6\text{H}_5\text{OH}$
Synthesis

(1) *From benzene sulphonic acid.*—Benzene is converted into its sulphonic acid. The sodium salt of the sulphonic acid is then fused with NaOH and Na-phenate is obtained. The free phenol is liberated with the help of a mineral acid and the phenol which separates out is purified by distillation:



(2) *From chlorobenzene.*—Chlorobenzene is heated to $350^\circ\text{--}380^\circ$ under very high pressure with NaOH solution in which some diphenyl ether is added and the chlorine atom is replaced by ONa. The phenol is liberated and purified by distillation:



Preparation from Coal-tar.—On a large scale, phenol is isolated from the *middle oil fraction* of coal-tar distillation which mostly consists of naphthalene and carbolic acid. On allowing the middle oil fraction to cool, most of the naphthalene crystallizes out. The oil which separates is treated with a warm caustic soda solution to form sodium phenate and any immiscible oil is separated mechanically. The caustic wash containing the soluble sodium phenate is then treated with dilute sulphuric acid; this liberates the phenol which separates as an oil. This is collected, washed with water and distilled. The distillate constitutes the crude carbolic acid of commerce. To obtain pure carbolic acid, the crude acid is fractionally distilled and the distillate crystallizes on cooling, the impure phenol being allowed to drain off.

Properties and Reactions.—Phenol crystallizes in colourless deliquescent prisms which melt at 43° and boil at 182° ; sp. gr. 1.072 at 20° . On exposure to air and light it turns pink. It has a very characteristic odour and is markedly caustic and poisonous. It is not very soluble in water (100 parts of water dissolve about 8.4 parts at 20°), but is freely soluble in alcohol and ether. It is volatile in steam.

It is acid in reaction, the H of the OH group being replaceable by metals, and it is hence called 'carbolic acid'. Thus, when treated with a caustic alkali it gives a *phenate*, e.g., $C_6H_5.ONa$. Phenol does not, however, react with an alkali carbonate which may be used to separate a phenol from an organic acid. Phenol gives with ferric chloride a **violet colour** which is discharged by alcohol and mineral acids. It is also recognised by Liebermann's reaction, and by the white crystalline precipitate of 2:4:6-*tribromo-phenol* $C_6H_2Br_3.OH$ with bromine water (m.p. 96°). With excess of bromine water, a precipitate of tribromophenyl hypobromite $C_6H_2Br_3.OBr$ is formed. For other tests see toxicology (pp. 447-450). When absorbed into the system phenol is excreted mostly conjugated with sulphuric acid or glycuronic acid.

Uses.—Phenol is largely used in surgery as a disinfectant both in the concentrated form and also in dilute solution. It is also used as a disinfectant powder mixed with kaolin.

Chemically, it is used in the manufacture of drugs such as salicylic acid, salol, aspirin, phenacetin and phenolphthalein, the explosive picric acid, certain azo dyes, and very largely in the preparation of synthetic plastics, such as Bakelite, etc.

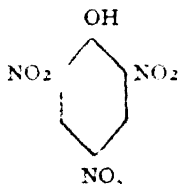
Phenolic Ethers.

Anisole, Phenyl methyl ether, $C_6H_5.O.CH_3$: obtained by heating an alcoholic solution of sodium phenate with methyl iodide, $C_6H_5.ONa + CH_3I = C_6H_5.O.CH_3 + NaI$; liquid with ethereal odour, b.p. 152° ; stable, neutral compound, like the *aliphatic ethers*, not readily hydrolyzed by acids or alkalis.

Phenetole, Phenyl ethyl ether, $C_6H_5.O.C_2H_5$: obtained by heating an alcoholic solution of sodium phenate with ethyl iodide, b.p. 172° ; properties similar to anisole.

Diphenyl ether, Diphenyl oxide, $C_6H_5.O.C_6H_5$: obtained by heating phenol with anhydrous aluminium chloride; crystalline solid m.p. 28° , b.p. 252° .

Picric acid, 2:4:6-Trinitro phenol, $C_6H_2(NO_2)_3.OH$



This acid is said to be formed when nitric acid acts on organic substances such as wool, leather, silk, resins, etc., as indicated by its yellow colour.

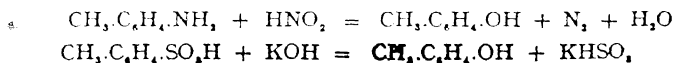
Preparation.—This is prepared by the nitration of phenol sulphonic acid, the latter being used instead of phenol in order to avoid the difficulty experienced in purifying picric acid from the tarry matter which is formed in the nitration of phenol. Phenol sulphonic acid is prepared by the action of strong sulphuric acid on phenol, the mixture being heated. It is then cooled, diluted with water and gradually added in small lots to strong nitric acid, shaking well between additions. When all the phenolsulphonic acid has been added, the mixture is heated on the water bath for two hours, a little fuming nitric acid being first added. The flask is then cooled and the picric acid separates out. It is purified by recrystallization from hot water.



Properties and Uses.—Picric acid crystallizes from hot water or alcohol in light yellow prisms, m.p. 122.5° , and possesses a very bitter taste. It is only slightly soluble in cold water (1.22 per cent at 20°), more readily in hot water and in alcohol, ether, chloroform, benzene, etc. Its solution dyes silk and wool yellow. It has a strong acid character and forms salts which crystallize well. The ammonium salt $C_6H_2(NO_2)_3 \cdot ONH_4$ is liable to explode if heated or struck. Picric acid itself is an explosive and should be mixed with water for storage. It forms crystalline compounds with naphthalene, anthracene, etc., and these picrates can be used for its identification. Picric acid is mainly used as an explosive. It is also used as an alkaloidal reagent.

Cresols, Hydroxy toluenes, $C_6H_4(CH_3)(OH)$

The three cresols (ortho-, meta-, and para-), which are homologues of phenol, occur in coal tar and in pine and beech wood tars, and are obtained during the fractionation of crude phenol. The crude phenol is distilled and the distillate crystallized out in the cold; the crystals of pure phenol are separated off by centrifuging from the fluid cresols. It is not, however, easy to isolate the individual cresols from this mixture and they are more easily prepared pure by synthetic methods, e.g., by treating the corresponding *toluidines* with nitrous acid or by fusing the corresponding toluene sulphonic acids with KOH:



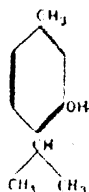
Their melting and boiling points are as follows: o-cresol, m.p. 31° , b.p. 190° ; m-cresol, m.p. 4° , b.p. 203° ; p-cresol, m.p. 36° , b.p. 202° .

The crude mixture of cresols obtained from coal tar or wood tar is utilized in making antiseptics and disinfectants such as *lysol*, *phenyle creolin*, etc., by making emulsions with resin soap or oil soap. The *B.P. cresol* is a mixture of o-, m-, and p. cresols and other phenols obtained from coal tar and having certain specific properties prescribed by it. The chlorine derivatives of cresols obtained by their

chlorination have a stronger antiseptic action than the cresols, and one of these, known as *chlorocresol* (6-chloro-3-hydroxy toluene) is used as a preservative and antiseptic. For tests see Toxicology (pp. 448-452).

Thymol, 1-Methyl-4-isopropyl-3-hydroxy benzene, $C_{10}H_{14}O$

This is found in the essential oil obtained from *ajowan* fruit, *Ptychotis ajowan* D.C. (*Carum copticum* B.Hk.). The oil, which constitutes about 2.5 per cent of the fruits, yields 35 to 45 per cent of thymol. The oil is shaken with a 5 per cent aqueous solution of NaOH which dissolves out the thymol. The aqueous solution is separated and free thymol liberated with the help of a mineral acid and distilled. It is purified by recrystallization from alcohol. It is also prepared synthetically.



Thymol gives transparent crystals, m.p. 50° , b.p. 233.5° (760 mm.). It has a thyme-like odour and a burning taste. It is very sparingly soluble in water (1 g. in 1176 c.c. at 20°), but is readily soluble in alcohol, ether, chloroform, oils, etc.

It is a powerful antiseptic and is employed in the treatment of intestinal parasites and also as a mouth wash.

An iodine derivative of thymol, diiododithymol known as *aristol* prepared by the action of iodine and KOH on thymol, is used as a substitute for iodoform.

DIHYDRIC PHENOLS

These exist in three isomeric forms; the ortho-compound is known as catechol, the meta-compound as resorcinol and the para-compound as quinol.



Catechol



Resorcinol



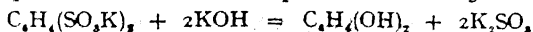
Quinol

Catechol, *Pyrocatechol*, *o*-Dihydroxy benzene, $C_6H_4(OH)_2$

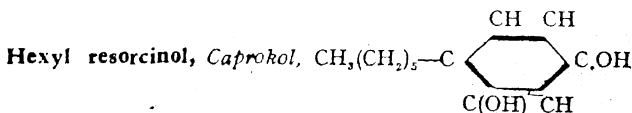
This occurs in catechu, the resin found in *Acacia catechu* Willd. It is prepared by fusing *o*-phenol sulphonic acid with caustic potash. It is a colourless crystalline solid, m.p. 104° , soluble in water, alcohol and ether. It sublimes in vacuo even at room temperature. With ferric chloride its aqueous solution gives an emerald-green colour which turns deep red with sodium carbonate or ammonia.

Resorcinol, *m*-Dihydroxy benzene, $C_6H_4(OH)_2$.

This is prepared by fusing the alkali salt of *m*-benzenedisulphonic acid with caustic potash to about 250° .



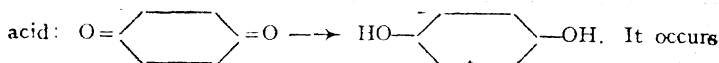
It forms colourless needles which become brown on exposure to air, m.p. 119° . It is soluble in water, alcohol and ether. Its aqueous solution gives a bluish violet coloration with ferric chloride which disappears on adding sodium bi-carbonate. It has antiseptic properties and is used in medicine in ointments and in throat paints. It is also used in making dyes like fluorescein, eosin, etc.



It is a strong antiseptic synthesized more recently and is used in medicine.

Quinol, Hydroquinone, *p*-Dihydroxy benzene, $C_6H_4(OH)_2$.

This is usually prepared by reducing quinone with sulphurous



in nature in the glucoside arbutin. It is a colourless crystalline solid, m.p. 169° . It is soluble in water, alcohol and ether, and is a strong reducing agent. It is employed in photography as a developer. A dilute aqueous solution gives a bluish-green colour with ferric chloride but the colour soon changes to brownish yellow.

TRIHYDRIC PHENOLS

Pyrogallol, Pyrogalllic Acid, 1:2:3-Trihydroxy benzene $C_6H_3(OH)_3$. It is obtained by heating gallic acid, and crystallizes in colourless needles, m.p. 132° . It is soluble in water, alcohol and ether, and sublimes without decomposition. It absorbs oxygen in alkaline solution and darkens in colour. A solution of pyrogallol in KOH is used in gas analysis for the estimation of free oxygen and in bacteriology for rendering the atmosphere of anaerobic bacteria oxygen free. It is also used in photography as a developer on account of its strong reducing action. With ferric chloride its aqueous solution gives a red colour and with ferrous sulphate and a trace of ferric chloride gives a deep blue colour.

Phloroglucinol, 1:3:5-Trihydroxy benzene $C_6H_3(OH)_3$. It is a colourless crystalline substance sweet in taste, m.p. 219° , soluble in water, alcohol and ether. With ferric chloride it gives a bluish violet colour.

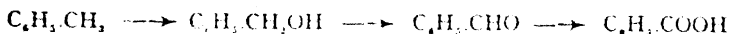
Hydroxyquinol, 1:2:4-Trihydroxy benzene $C_6H_3(OH)_3$. Crystalline substance, m.p. 140° , soluble in water. Ferric chloride gives a greenish-brown colour, which changes to blue and then to red on the addition of sodium bicarbonate.

CHAPTER XXV

AROMATIC ALCOHOLS, ALDEHYDES, KETONES AND ACIDS

AROMATIC ALCOHOLS

A true aromatic alcohol is derived from an aromatic hydrocarbon by the replacement of a hydrogen atom of an alkyl radical of the *side chain* by an OH group and thus differs from a phenol. As might be expected the behaviour of the aromatic alcohols is analogous to that of the aliphatic alcohols; hence we have alcoholates, esters, ethers, amines, etc., the oxidation of the alcohol to aldehyde and acid and reduction of the aromatic acids, aldehydes and ketones to the alcohols:

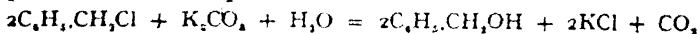


Benzyl Alcohol, *Phenyl carbinol*. $\text{C}_6\text{H}_5\text{CH}_2\text{OH}$

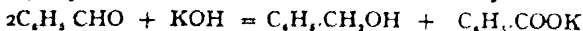
Occurrence.—This is found as an ester of cinnamic acid and benzoic acid in Balsam of Tolu and Peru and in Storax, the exudates obtained from the trees *Myroxylon toluiferum* Hb.K., *M. pereirae* Kltsch, and *Liquidambar orientalis* Mill. respectively and used in medicine.

Preparation

(1) By the hydrolysis of benzyl chloride with an aqueous solution of potassium carbonate:

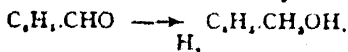


(2) By the action of KOH on benzaldehyde:

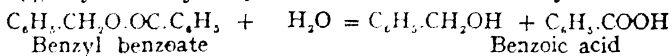


This reaction by which an alcohol and an acid are produced from an aldehyde is known as *Cannizzaro reaction*, and is characteristic of aromatic aldehydes.

(3) By the reduction of benzaldehyde:



(4) By the hydrolysis of an ester of benzyl alcohol:



The first and second reactions are mainly used for the industrial preparation of benzyl alcohol.

Properties and Reactions.—It is a colourless liquid, b.p. 205° , with a faint aromatic smell; sp. gr. 1.042 at 20° . It is only slightly soluble in water but easily soluble in alcohol and ether. On oxidation it is first converted into benzaldehyde and finally into benzoic acid. Conc. HCl converts it into benzyl chloride $\text{C}_6\text{H}_5\cdot\text{CH}_2\text{Cl}$, and conc. HNO_3 converts it into benzaldehyde; these reactions and the action of caustic alkali distinguish benzyl alcohol from the isomeric cresols (see p. 305).

Cinnamic Alcohol, $\text{C}_6\text{H}_5\cdot\text{CH}:\text{CH}\cdot\text{COOH}$: This is a type of an aromatic *unsaturated* alcohol, and the corresponding acid cinnamic acid (see) is known to occur in nature.

AROMATIC ALDEHYDES

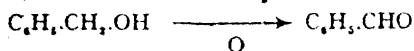
When the CHO group is directly linked to a carbon atom of the nucleus it is usual to call this an aromatic aldehyde. When, however, the aldehyde group occurs in the side chain, such an aldehyde shows the properties of an aliphatic aldehyde. Unlike the aliphatic aldehydes, the aromatic aldehydes do not polymerize nor resinify with caustic alkalies but they give the Cannizzaro reaction instead. They do not undergo aldol condensation but show the benzoin condensation with alcoholic KCN (see benzaldehyde). They do not reduce Fehling's solution but ammoniacal silver nitrate is reduced slowly.

Benzaldehyde, *Oil of Bitter Almonds*, $\text{C}_6\text{H}_5\cdot\text{CHO}$

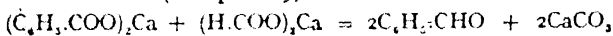
Occurrence.—This occurs in nature in the glucoside amygdalin found in bitter almonds (hence Oil of Bitter Almonds). When amygdalin is boiled with a dilute mineral acid, it yields benzaldehyde, HCN and glucose (see p. 104). When bitter almonds are crushed in a mortar with water, amygdalin is hydrolyzed by the enzyme present and the odour of benzaldehyde becomes apparent.

Preparation

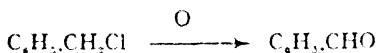
(1) By the oxidation of benzyl alcohol with conc. HNO_3 :



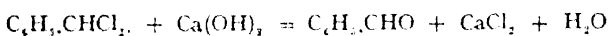
(2) By distilling a mixture of calcium benzoate and calcium formate (see p. 109):



(3) By the oxidation of benzyl chloride with an aqueous solution of $\text{Cu}(\text{NO}_3)_2$:



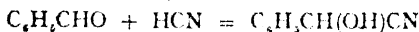
(4) By heating benzal chloride with milk of lime under pressure:



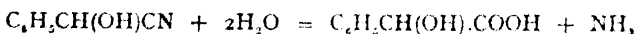
The last two methods are used on a large scale for its manufacture.

Properties and Reactions.

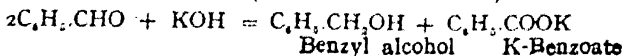
Benzaldehyde is a colourless liquid with a bitter almond odour; sp. gr. 1.041 at 25° , b.p. 175° , volatile in steam. It is only slightly soluble in water but easily soluble in alcohol and ether. On exposure to the air it is gradually oxidized to benzoic acid which separates out as a crystalline solid. On reduction it yields benzyl alcohol. Like aliphatic aldehydes it gives Schiff's reaction (see pp. 80, 111), combines with sodium bisulphite, phenylhydrazine and hydroxylamine. It does not, however, form an aldehyde-ammonia and does not reduce Fehling's solution. When treated with HCN benzaldehyde yields the cyanhydrin known as *mandelonitrile*:



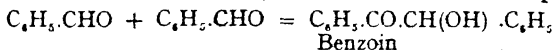
On hydrolysis, mandelonitrile gives *mandelic acid* which is used in medicine for the treatment of *B. coli* infection.



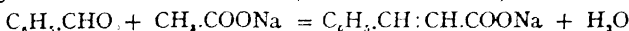
It gives a mixture of benzyl alcohol and benzoic acid when treated with KOH (*Cannizzaro reaction*):



On boiling with an alcoholic solution of KCN it undergoes *benzoin condensation* (cf. aldol condensation, p. 116):



When heated with sodium acetate and acetic anhydride, it gives sodium cinnamate (*Perkin's reaction*):



Cinnamic Aldehyde, $C_6H_5.CH:CH.CHO$: This is a type of an aromatic unsaturated aldehyde, and is the chief constituent of oil of cinnamon, the essential oil obtained from *Cinnamomum zeylanicum* Nees.; liquid, b.p. 247° with an odour of cinnamon.

AROMATIC KETONES

These may be *true* aromatic ketones, *viz.*, containing two aryl groups such as benzophenone $C_6H_5.CO.C_6H_5$, etc, or they may be *mixed*, *viz.*, containing one aryl group and one alkyl group, such as acetophenone, $C_6H_5.CO.CH_3$, etc.

Benzophenone, Diphenyl Ketone, $C_6H_5.CO.C_6H_5$: This may be prepared by distilling calcium benzoate; crystalline solid, m.p. 48° , b.p. 305.4° .

Acetophenone, Phenyl methyl ketone, $C_6H_5.CO.CH_3$: This may be prepared by distilling a mixture of calcium benzoate and calcium acetate; it crystallizes in colourless plates, m.p. 20.5° , b.p. 202° ; sp. gr. 1.026 at 25° ; soluble in water and alcohol and has a peculiar smell; it is sometimes used in medicine as a hypnotic under the name *hypnone*.

The chlorine derivative of acetophenone, *chloro acetophenone* $C_6H_5.CO.CH_2Cl$, known as C.A.P. was used as a "lachrymatory gas" during the first Great War.

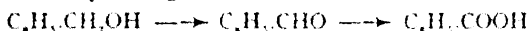
AROMATIC MONOBASIC ACIDS

Two classes of aromatic acids may be distinguished: (1) When a hydrogen atom of the nucleus is substituted by a carboxyl group, *e.g.*, benzoic acid, (2) when a hydrogen atom in the side chain is substituted by a carboxyl group, *e.g.*, phenyl acetic acid $C_6H_5.CH_2.COOH$.

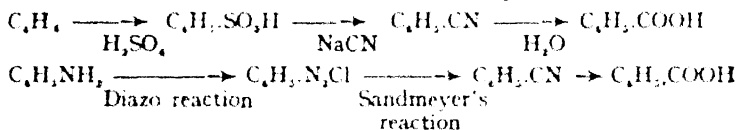
In most respects the aromatic acids are analogous to the aliphatic acids. They form salts with metals, esters with alcohols, and give chlorides, amides, etc. When heated with soda lime they give off carbon dioxide and form the corresponding hydrocarbons.

General Methods of Preparation

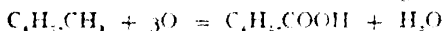
(1) By the oxidation of the corresponding aromatic alcohol or aldehyde *e.g.*,



(2) By the hydrolysis of the corresponding nitriles; these nitriles can be obtained from the sulphonates or amines:



(3) By the oxidation of benzene homologues:



Benzoic Acid, C_6H_5COOH

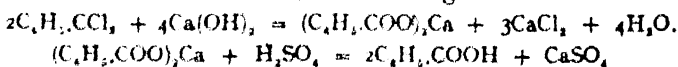
Occurrence.—In the free state as well as in combination it occurs in certain resins, especially *gum benzoin*, a resin obtained from *Styrax benzoin* Dryand, found in Java, Sumatra, and other places. It can be obtained by heating the gum benzoin when the benzoic acid sublimes. In the urine of herbivora, it occurs as hippuric acid or benzoyl glycine $C_6H_5CO.NH.CH_2.COOH$ from which the acid can be obtained by hydrolysis.

Synthesis

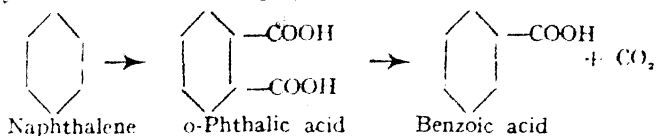
- (1) Oxidation of benzyl alcohol or benzaldehyde.
- (2) Hydrolysis of benzonitrile.
- (3) Oxidation of toluene.

Preparation

(1) By the hydrolysis of benzotrichloride, obtained by the chlorination of toluene, with boiling milk of lime:



(2) By the decarboxylation of the phthalic acid obtained by the oxidation of naphthalene:



Properties and reactions

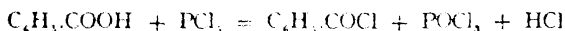
Benzoic acid crystallizes from hot water in colourless shining flakes. It melts at 121.5° and is volatile in steam. It sublimes easily and its vapour produces coughing and sneezing. It is only sparingly soluble in cold water (1 in 372 at 17.5°) but more easily soluble in hot water, alcohol and ether. With ferric chloride it gives a pale brown or buff coloured precipitate of ferric benzoate. When heated with soda lime, benzene and carbon dioxide are formed: $\text{C}_6\text{H}_5\text{COOH} \longrightarrow \text{C}_6\text{H}_6 + \text{CO}_2$. On heating benzoic acid with ethyl alcohol in presence of dry HCl gas *ethyl benzoate* $\text{C}_6\text{H}_5\text{COOC}_2\text{H}_5$ is formed; this ester has a peculiar aromatic odour and is used for the identification of benzoic acid.

Benzoic acid, like the aromatic hydrocarbons generally, is readily acted upon by nitric acid and sulphuric acid forming nitro-benzoic and sulphobenzoic acids. It may also be acted on by chlorine or bromine forming chlorobenzoic and bromobenzoic acids.

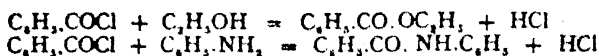
Benzoic acid and its salts are used in medicine as diuretic, antiseptic and expectorant.

Benzoyl Chloride, $\text{C}_6\text{H}_5\text{COCl}$

This is an *acid chloride* analogous to acetyl chloride and may be formed by the action of phosphorus pentachloride on benzoic acid.

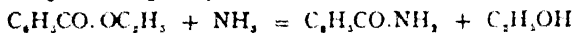


It is a colourless oily liquid with an irritating odour. It is used for the detection and isolation of hydroxy and amino-compounds, as it reacts forming benzoyl derivatives which are mostly crystalline. The H atom is replaced by benzoyl group $\text{C}_6\text{H}_5\text{CO}$ —and the process is known as *benzoylation* (cf. acetylation, p. 148).



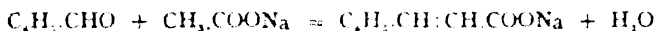
Benzamide, $\text{C}_6\text{H}_5\text{CONH}_2$

This is an *acid amide* analogous to acetamide and can be prepared by heating ethyl benzoate with ammonia:



It is a colourless crystalline solid, m.p. 130° , soluble in hot water, alcohol and ether. On boiling with acids it decomposes into benzoic acid and ammonia.

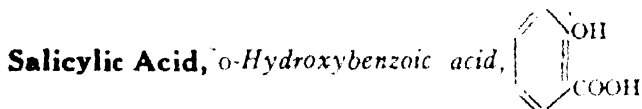
Cinnamic Acid, $\text{C}_6\text{H}_5\text{CH}:\text{CH}\cdot\text{COOH}$: This is an example of an *unsaturated* aromatic acid found as ester as well as in the free state in Balsams of Tolu and Peru as also in Storax. It can be prepared synthetically by *Perkin's reaction* by heating benzaldehyde with anhydrous sodium acetate and acetic anhydride:



It is a colourless crystalline solid, m.p. 133° , soluble with difficulty in cold water but more easily in hot water and in alcohol and ether. As an unsaturated acid it combines with bromine and halogen acids.

HYDROXY ACIDS

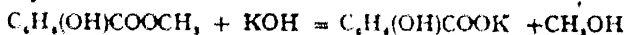
Hydroxy acids may be classified as those with
(1) carboxyl group in the nucleus, e.g., salicylic acid, and
(2) COOH group in the side chain, e.g., mandelic acid.



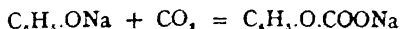
Occurrence.—Found as methyl ester as the main constituent of *Oil of Wintergreen* obtained from the leaves of *Gaultheria procumbens* L. or from the bark of *Betula lenta* L.

Preparation

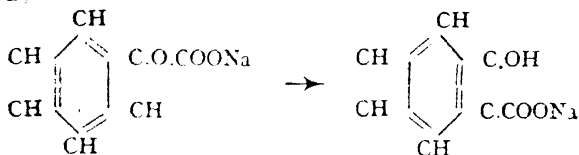
(1) *From Oil of Wintergreen.* The ester is hydrolyzed by heating with a dilute solution of KOH , the methyl alcohol formed is distilled off and the salicylic acid is liberated by dilute sulphuric acid. On concentration of the aqueous solution salicylic acid crystallizes out, and is purified by recrystallization from hot water:



(2) *From Phenol (Kolbe's Reaction).*—Dry sodium phenate is heated to 120° - 140° and treated with carbon dioxide gas in a closed vessel under pressure. Sodium phenyl carbonate is first formed:



This compound then undergoes intramolecular change at the high temperature, the COONa group replacing an ortho hydrogen atom of the nucleus and sodium salicylate is formed:



The sodium salt is decomposed by dilute sulphuric acid, the salicylic acid is obtained by concentration and finally purified by recrystallization from hot water.

Properties, Reactions and Uses. Salicylic acid crystallizes in colourless needles, and has a sweetish taste, m.p. 155° . It is only slightly soluble in cold water (100 parts of water dissolve 0.184 g. at 20°) but more readily soluble in hot water, alcohol and ether. It is volatile with steam. It sublimes on slow heating but if rapidly heated it decomposes into phenol and carbon dioxide. With ferric chloride a violet colour is produced which is discharged by mineral acids but not by alcohol (cf. phenol, p. 303). With bromine water, it gives a yellowish white precipitate of tribromosalicylic acid which is soluble in alcohol. For other tests see Toxicology (p. 512).

It is a strong antiseptic and is used as an antifermenting agent. It softens the epidermis and is, therefore, an important constituent of all "corn cures." Its sodium salt, sodium salicylate, is most valuable in the treatment of rheumatic fever.

Para-hydroxy benzoic acid (m.p. 210°) is used as a food-preservative. It is not volatile with steam.

Aspirin, Acetyl salicylic acid, $C_6H_4(O.COCH_3)COOH$: This is prepared by heating salicylic acid with acetic anhydride. Aspirin is a white crystalline substance easily soluble in alcohol but sparingly in water; m.p. 135° ; gives a yellow colour with ferric chloride since the phenolic group is acetylated. It is used in medicine as an analgesic and antipyretic. For solubility, tests, &c., see pp. 513-514.

Salol, Phenyl salicylate, $C_6H_4(OH).COOC_6H_5$.

This is prepared by heating a mixture of sodium salts of salicylic acid and phenol with phosphorus oxychloride to about 120° . It is a crystalline solid, m.p. 43° . It is used as an intestinal antiseptic, being hydrolyzed in the intestines to phenol and salicylic acid.

Gallic Acid, 3:4:5—Trihydroxy benzoic acid, $C_6H_2(OH)_3COOH$.

This occurs in gall nuts and in many tannins, from which it may be obtained by hydrolysis with dilute acids. It crystallizes in needles, m.p. 225° . It is not very soluble in cold water, but soluble in hot water, alcohol and ether. On heating it decomposes into carbon dioxide and pyrogallol. It dissolves in aqueous alkalis but the solution turns brown rapidly due to oxidation.

With ferric chloride it gives a bluish-black colour or precipitate. It does not precipitate gelatin from solution and is thus distinguished from tannic acid. It is used for the preparation of ink. A basic bismuth salt of gallic acid, known as *dermatol*, is used as an antiseptic in skin diseases.

Tannins.—These are widely distributed in plants. They are soluble in water and possess an astringent taste. They precipitate gelatin solution forming an insoluble compound and for this reason they are utilized in the manufacture of leather, as the insoluble compound formed does not undergo putrefactive changes. The tannins principally used in tanneries are derived from myrobalans (*hari'aki*), sumach, catechu, acacia (*babul*), oak, etc. Tannins give a blue-black or green colour with ferric salts hence they are used for making inks. Tannins are also precipitated by lead acetate, albumen and by

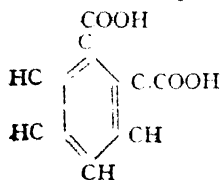
alkaloids. They are used in medicine as astringents and styptics.

The structure of many of the tannins has not yet been elucidated but a large number appear to be esters of glucose with *polyhydroxy benzoic acids*, such as gallic acid, etc.

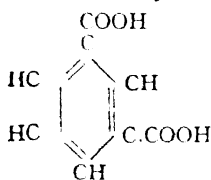
Tannic acid, Gallotannic Acid.—This is a tannin which occurs in oak-galls, in tea, in catechu, etc., and is found as colourless or yellowish amorphous mass. It is easily soluble in water and dilute alcohol but not in absolute alcohol or ether. With gelatin solution it forms a white precipitate and with ferric chloride it gives a blue black colour or precipitate. On hydrolysis with 2% HCl it yields gallic acid. It is now used extensively in the treatment of burns and as an astringent in throat pains.

Aromatic Dibasic Acids,

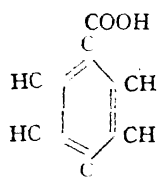
In these acids both the carboxyl groups may be attached to the carbon atoms of the benzene nucleus as in phthalic acid $C_6H_4(COOH)_2$ or both the carboxyl groups may remain in the side chains, *e.g.*, $C_6H_4(CH_2COOH)_2$, or one may remain in the nucleus and one in the side chain, *e.g.*, $C_6H_4(COOH).CH_2COOH$. Of the three isomers of benzene dicarboxylic acid, which can be obtained by the oxidation of the corresponding xylenes, the ortho-compound is the most important economically.



Phthalic acid
(1:2—Benzene
dicarboxylic
acid)



Isophthalic acid
(1:3—Benzene
dicarboxylic
acid)

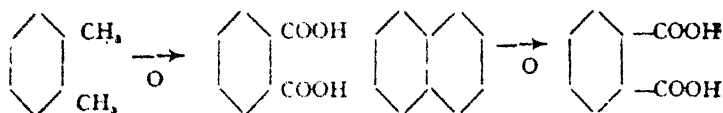


Terephthalic acid
(1:4—Benzene
dicarboxylic acid)

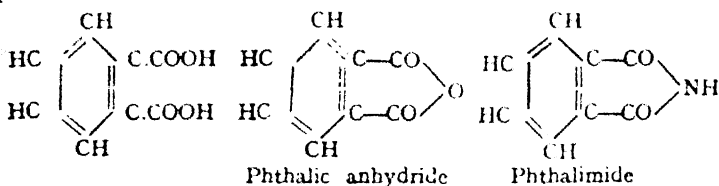
Phthalic Acid, Benzene -o-dicarboxylic acid,



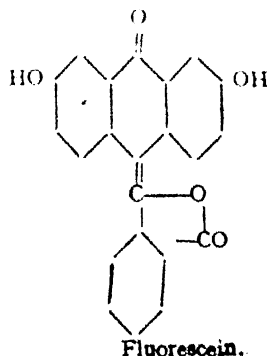
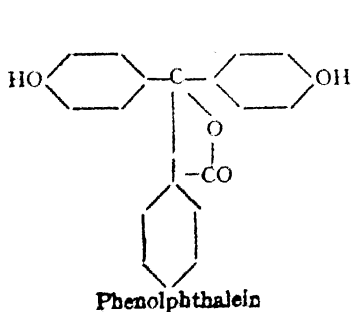
It can be prepared by the oxidation of o-xylene; it is usually prepared by the oxidation of naphthalene by heating with fuming sulphuric acid in presence of mercury as catalyst at about 250° .

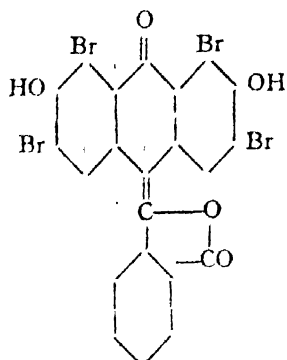


Phthalic acid crystallizes in colourless plates, m.p. 213° , soluble in water, alcohol and ether. When heated above its melting point it gives phthalic anhydride. When phthalic anhydride is heated with anhydrous ammonia, it gives phthalimide:

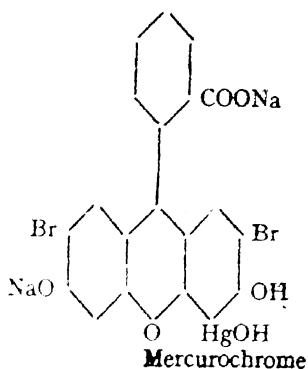


The acid potassium salt of phthalic acid, $C_6H_4(COOH)COOK$, is used as a *buffer* in pH work. Phthalic anhydride is used in the preparation of the indicator and purgative principle *phenolphthalein*. It is also used for the synthesis of various dyestuffs such as *fluorescein* (used in eye surgery), *eosin* (tetrabromofluorescein), *mercurochrome 220* (di-sodium salt of 2:7-dibromo-4-hydroxy-mercury fluorescein), *indigo*, *erythrosin*, etc., as well as in the manufacture of some synthetic plastics. An important derivative of phenolphthalein, sodium tetraiodophenolphthalein, is used in X-ray work for rendering the gall-bladder opaque.





Tetrabromofluorescein;
its Na-salt is Eosin

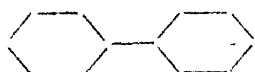


Mercurochrome

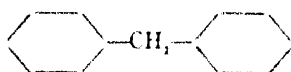
CHAPTER XXVI

NAPHTHALENE, ANTHRACENE, ETC., AND SOME HETEROCYCLIC COMPOUNDS

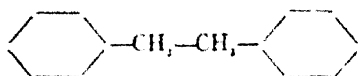
We have so far considered *carbocyclic compounds* containing only one closed ring system, viz., that of benzene. Aromatic compounds with *two or more* benzene nuclei also exist and the structure of some of these are shown below:



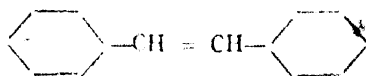
Diphenyl



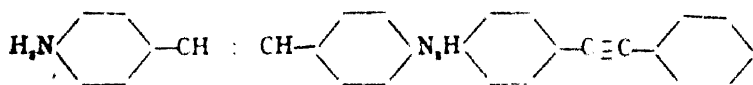
Diphenyl methane



Dibenzyl
(Diphenyl ethane)

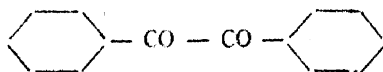


Stilbene
(Diphenyl ethylene)

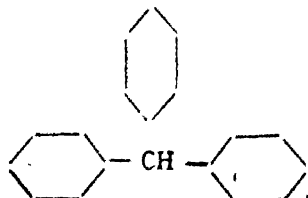


p-Diamino stilbene

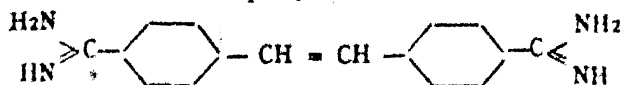
Tolane
(Diphenyl acetylene)



Benzil



Triphenyl methane



4,4'-Diamidostilbene

There are, however, other carbocyclic ring systems which contain more than one benzene ring fused together. These are known as *condensed ring nuclei*. Examples of these are naphthalene, anthracene and phenanthrene, which occur in coal tar and form the basis of many synthetic dye-stuffs. The parent hydrocarbons behave like benzene, yielding a large variety of similar derivatives.

Naphthalene, $C_{10}H_8$

Preparation.—This hydrocarbon is obtained from the middle or carbolic fraction of coal tar distillation by allowing it to cool, when the impure naphthalene separates out; this is pressed to free from adhering liquids and washed with caustic soda to remove phenols. It is subsequently treated with strong sulphuric acid to remove other impurities as soluble sulphonic acid compounds, again washed and finally sublimed or distilled in steam.

Properties, Reactions and Uses.—Naphthalene crystallizes in colourless, shining plates, m.p. 80° , b.p. 218° . It is insoluble in water but soluble in ether and alcohol. It is extremely volatile, and often causes blocking in gas mains due to this property. It has a characteristic smell and burns with a very smoky flame. When dissolved in an alcoholic solution of picric acid it forms a *picrate* which crystallizes in yellow needles, m.p. 149° .

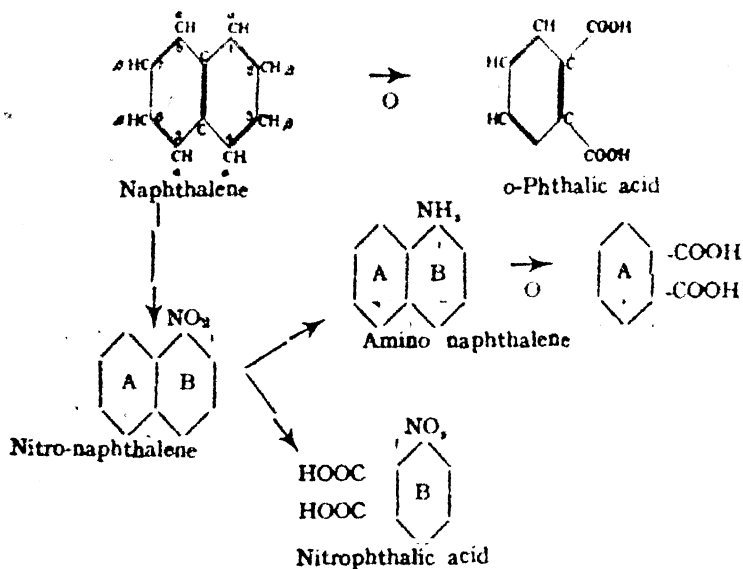
Naphthalene is largely used as an insecticide as moth balls but its principal chemical use is in the manufacture of various synthetic dyes such as indigo, azo dyes, etc.

In its behaviour naphthalene closely resembles benzene, yielding nitro and sulphonic acid compounds; with chlorine, it gives additive compounds such as naphthalene tetrachloride $C_{10}H_8Cl_4$, etc.

Structure of Naphthalene

The molecular formula of naphthalene is $C_{10}H_8$. It contains a benzene nucleus as proved by its oxidation with dilute nitric acid to ortho-phthalic acid, which consists of a benzene ring having two COOH groups in ortho position. If nitronaphthalene is oxidized, it gives nitrophthalic acid, the ring containing the nitro group remaining intact. If the

nitro-naphthalene is reduced, it forms amino-naphthalene or naphthylamine which on oxidation gives orthophthalic acid, the ring containing the amino group being destroyed giving rise to two COOH groups. These facts point to the existence of two condensed six-membered rings and the structural formula can be best represented as follows:



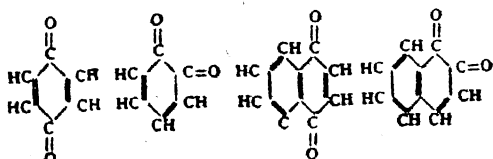
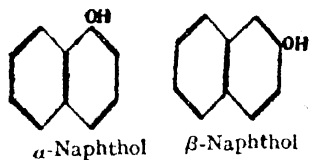
Isomerism of Naphthalene Compounds.—Of the eight hydrogen atoms all are not similarly placed relatively to the rest of the molecule, one set of four occupying positions different from the other set of four. These two positions are usually distinguished by the letters α and β ; hence we have two mono derivatives in which substituents may exist in the α or β position. The different positions of the H atoms are also represented numerically (see diagram above). Of the disubstitution products, ten isomeric derivatives may exist, their position being generally designated by numerals such as 1:2, 1:3, 2:3, 2:6, etc. The position 1:2, 1:3 and 1:4 in the same ring are known as ortho-, meta- and para-positions, while the position 1:8 is termed the *peri*-position.

Derivatives of Naphthalene

The *halogen* derivatives are readily prepared but they are of little importance. Naphthalene is easily nitrated; the α -nitronaphthalene is converted on reduction into α -naphthylamine $C_{10}H_7.NH_2$ (cf. aniline), which is used as a reagent and also in the manufacture of dyes. Naphthalene also reacts with concentrated sulphuric acid forming naphthalene *sulphonic acids* $C_{10}H_7.SO_3H$, which are utilized largely in the dye industry.

The α - and β *monohydroxy* derivatives of naphthalene are important as they constitute the two naphthols. They may be obtained from naphthalene sulphonic acids by fusing with caustic potash (cf. phenols) or by diazotizing the naphthylamines. Both are crystalline substances with a phenolic odour. β -Naphthol, $C_{10}H_7.OH$, is a crystalline solid, m.p. 123° . It is used internally as an intestinal antiseptic. α -Naphthol is a crystalline solid, m.p. 96° . It is also an antiseptic but is more irritating. Both the naphthols give colour reactions with ferric chloride.

If two hydrogen atoms of the benzene nucleus are replaced by two oxygen atoms, the products are called quinones or benzo-quinones. Similarly naphthalene yields quinones which are known as naphtho-quinones. The structures of these quinones are given below:



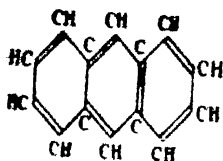
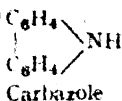
p-Benzoquinone o-Benzoquinone α -or 1:4-naphthoquinone β -or 1:2-naphthoquinone

These naphthaquinones are prepared by the oxidation of the

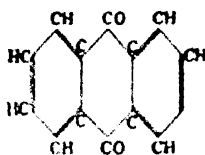
corresponding dihydroxy naphthalenes, and have properties similar to the benzo-quinones. Vitamins K are derivatives of α -naphthaquinone (see pp. 393—394).

Anthracene, $C_{14}H_{10}$

This is a hydrocarbon found in the distillate from coal tar in the fraction above 270° , known as the anthracene oil fraction. This fraction is distilled again and washed with solvent naphtha. The brown mass obtained is again distilled with potassium carbonate to remove carbazole and the distillate which contains anthracene and phenanthrene is treated with carbon disulphide to remove the phenanthrene. Anthracene crystallizes from benzene in colourless plates which show a blue fluorescence, m.p. 216° . It is insoluble in water and only very slightly soluble in alcohol; it is soluble in benzene. With picric acid it gives a compound which crystallizes in red needles, m.p. 138° . With chlorine and bromine anthracene reacts similarly to naphthalene; with sulphuric acid, sulphonic acid compounds are formed; with nitric acid, however, instead of nitro compounds being formed the anthracene is oxidized to anthraquinone. Actually in practice most oxidizing compounds convert anthracene into anthraquinone.



Anthracene



Anthraquinone

The general importance of anthracene or anthraquinone lies in the fact they are the mother substances of a large number of dyes, e.g., alizarin, etc.

Alizarin, 1:2-Dihydroxy anthraquinone $C_{14}H_8O_4$

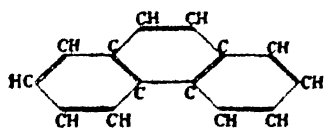
This is found in madder root (*Rubia tinctorum* L.), which owes its dyeing properties to alizarin and purpurin, both occurring as glucosides in the root. Dyeing by madder root is now practically obsolete, as alizarin itself is made on a large scale from anthraquinone- β -sulphonic acid by fusing it with potassium hydroxide and chlorate. Alizarin crystallizes in red crystals, m.p. 289° , insoluble in water but soluble in alcohol and ether and also in dilute alkalis, giving a reddish violet solution.

These soluble compounds combine with oxides of Al, Fe, Cr, etc., to form insoluble alizarates of different colours called lakes. Cotton fibres are dyed on this principle. They are first generally treated with an acetate of Al, Fe, Cr, etc., and then submitted to heat by

which the corresponding oxide is left in the fibre and the cotton is said to be *mordanted*. When the mordanted fibre is next treated with a soluble alizarate the oxide unites with the alizarin forming an insoluble alizarate and the cotton is dyed permanently.

Phenanthrene, $C_{14}H_{10}$

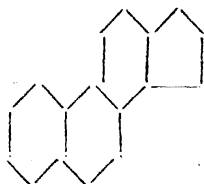
This is an isomeric of anthracene found in coal tar. It



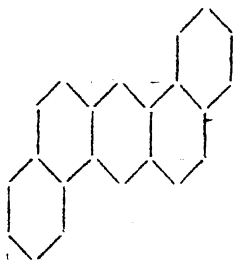
crystallizes in lustrous needles, m.p. 99° . It is soluble in ether and benzene but less readily in alcohol, glacial acetic acid, etc. With picric acid it yields a yellow crystalline compound, m.p. 143° .

When oxidized with chromic acid it yields *phenanthraquinone*, $C_{14}H_8O_2$.

It is of interest to remember that a complex phenanthrene nucleus, known as the *cyclopentenophenanthrene* skeleton (see Fig.), is present in various compounds of medical interest, e.g., in *sterols* such as cholesterol, ergosterol, etc., in *vitamins D*, in bile acids as cholic acid, etc., in *sex hormones* such as oestrone, testosterone, cortin, etc. Again, a complex anthracene nucleus, known as the *dibenzanthracene* skeleton (see Fig.), is found in compounds which are believed to produce cancer.



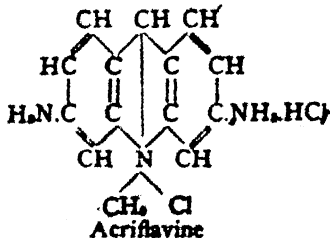
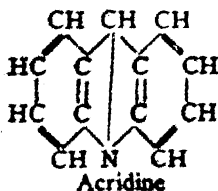
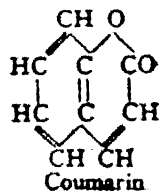
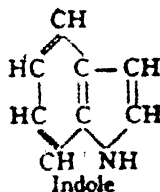
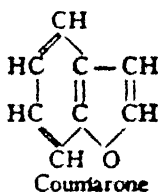
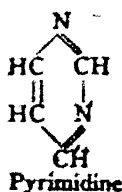
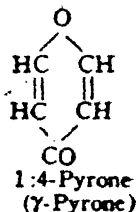
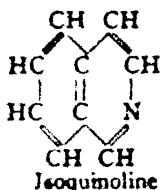
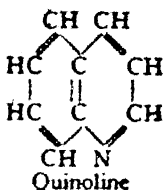
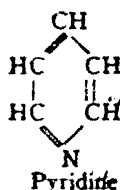
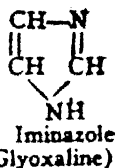
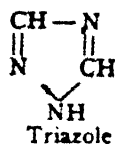
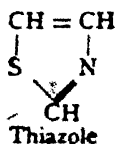
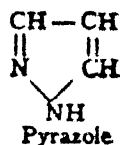
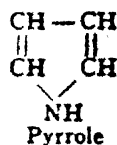
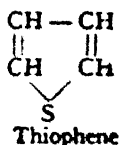
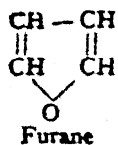
Cyclopentenophenanthrene,
skeleton



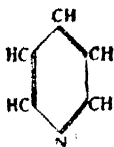
Dibenzanthracene
skeleton

Heterocyclic Compounds

We have so far dealt with only *carbocyclic compounds*, i.e., compounds in which only carbon atoms have taken part in the ring formation. There are, however, a large number of compounds in which one or more C atoms of a ring may be replaced by other polyvalent atoms such as O, S, N, etc., and these are known as *heterocyclic compounds*. Some of them (like the alkaloids) occur in plants, others such as histidine, proline, tryptophane, etc., occur in the animal kingdom, and a host of others have been prepared synthetically. The structures of a few are shown here:



(2:8-Diamino-10-methyl) acridinium chloride)

Pyridine C_5H_5N *Occurrence and Preparation.*—

Pyridine occurs in small quantities in coal tar. It is also found in *bone oil* (*Dippel's oil*) which is obtained by the dry distillation of bones. It may be prepared from the coal tar fraction boiling between 80° and 250° and the bases are separated by washing with dilute sulphuric acid. The acid liquid is separated and the bases liberated by NaOH. Pyridine is then separated from the mixture of bases by fractional distillation.

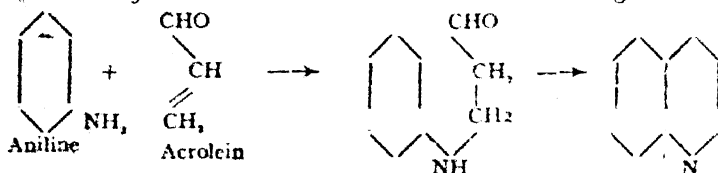
Properties and Reactions.—Pyridine is a colourless liquid with a characteristic unpleasant odour, b.p. 115.5° . It has a sp. gr. of 0.989 at 15° . It is easily soluble in water, alcohol and ether. It is a strong base with alkaline reaction and behaves like NH_3 , forming white fumes of pyridine hydrochloride when it is exposed to the fumes of strong HCl. It also forms salts with other acids. It is a very stable substance and gives a characteristic crystalline precipitate with mercuric chloride. Nitric acid and potassium permanganate do not attack it but conc. sulphuric acid yields a sulphonie acid derivative. It is a tertiary base and on treatment with sodium and alcohol, pyridine is reduced to the secondary base, *piperidine* $C_4H_{11}N$, a constituent of the alkaloid piperine found in black pepper. Pyridine is used as a solvent and is also used in this country for denaturing spirit (0.5 per cent).

Derivatives of Pyridine. The alkyl derivatives, *e.g.*, the methyl pyridines or picolines $C_5H_4N.CH_3$, are also found in coal tar or bone oil. These picolines can be oxidized by $KMnO_4$ to pyridine carboxylic acids $C_5H_4N.CO.OH$ (see nicotinic acid). An important derivative of pyridine- β -carboxylic acid is its diethylamide, known as **coramine** $C_5H_4N.CO.N(C_2H_5)_2$, which is used in medicine as a cardiac stimulant.

Quinoline, C_9H_7N

It is a benzopyridine and may be considered as naphthalene in which one CH group is replaced by N. It occurs in coal tar and in bone oil and is isolated from them

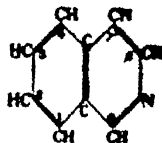
as described above for pyridine. It can also be obtained by distilling many alkaloids, particularly the cinchona alkaloids. It is generally prepared synthetically by *Skraup's reaction*, which consists in heating a mixture of aniline, glycerol and strong sulphuric acid in presence of arsenic acid or nitrobenzene as oxidising agent. The glycerol is converted into acrolein which reacts with aniline; the condensed product is then oxidized by arsenic acid with the closure of the ring:



Quinoline is a colourless oily liquid with a characteristic smell, b.p. 238° , sp. gr. 1.095 at 20° . It is sparingly soluble in water but dissolves easily in alcohol, ether and benzene. It is a tertiary base and forms salts with acids. The quinoline ring is present in some alkaloids, e.g., cinchonidine, quinine, &c. Quinoline has been the basis of several synthetic drugs such as *chiniton* (7-iodo-8-hydroxy quinoline-5-sulphonic acid), *entero-vioform* (iodochloro hydroxy quinoline), *pamaquin*, etc.

Isoquinoline, C_9H_7N

This is an isomer of quinoline and occurs in coal tar and bone oil. It is a colourless liquid with a characteristic smell, b.p. 241° . It is a tertiary base and forms salts with acids. The isoquinoline ring is present in some alkaloids, e.g., morphine, emetine, etc.



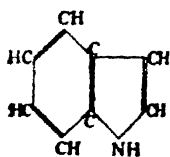
Pyrrole, Pyrrol, C_4H_5N

The importance of this compound lies in the fact that the pyrrole ring enters into the structure of haemoglobin, of chlorophyll, of bilirubin and of some alkaloids. Pyrrole occurs in bone oil and to a smaller extent in coal tar. It is isolated from bone oil by fractional distillation. It is a colourless liquid, turning brown in air, with a chloroform-like odour and boils at 131° . It is sparingly soluble in water but dissolves easily in alcohol and ether. It is a weak secondary base soluble in aqueous alkalis and dissolves slowly in



dilute acids. If the acid solution is heated, a red flocculent precipitate giving an amorphous powder, known as *pyrrole red*, is obtained. The vapour of pyrrole when allowed to act on a pine shaving moistened with HCl colours it red, whence the name (*pyrros*, red). It combines with picric acid to form an unstable picrate. On reduction, pyrrole is converted into *pyrrolidine* C_4H_7N , a ring structure found in the amino acid, proline. When treated with metallic potassium, pyrrole forms a solid potassium compound C_4H_5NK , the K replacing the H atom attached to N. An iodine derivative of pyrrole, known as Iodol C_4I_2NH (*tetraiodopyrrol*), an odourless and non-irritant compound, is used as an antiseptic as a substitute for iodoform.

Indole, Indol, Benzopyrrole, C_8H_7N

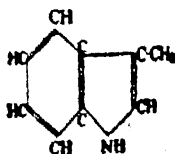


This substance is present in faeces, being a product of protein putrefaction. The characteristic faecal odour is due to the presence of indole and skatole. It is also present in jasmine flower oil (from *Jasminum grandiflorum* L.) to the extent of nearly 2.5 per cent from which it can be prepared, as well as in orange blossoms and in other flowers.

If it is carefully purified and mixed in suitable concentration with other perfumes, a surprisingly sweet odour of fresh flowers is imparted to the mixture, this property being utilized in the perfume industry.

It crystallizes in bright colourless plates, m.p. 52° . It is volatile in steam and is fairly soluble in hot water, alcohol, ether, etc. It is a weak secondary base forming salts with acids. On oxidation, indole is converted into indoxyl, a compound which appears in the urine as its ethereal sulphate (see below). Indole is obtained by the decomposition of the amino acid tryptophane by some bacteria and hence this reaction is utilized in bacteriology to identify these organisms. There are several *colour tests* for indole; e.g., (1) an alcoholic solution of p-dimethylamino-benzaldehyde is mixed with the indole solution and conc. HCl added drop by drop, a red colour is produced (Ehrlich); (2) the solution of indole is treated with a little formaldehyde and conc. H_2SO_4 allowed to run in by the side, a violet colour is produced at the junction (Kondof); (3) a solution of sodium nitroprusside is mixed with the indole solution and a few drops of NaOH added, a violet blue colour is produced which is changed to blue by acetic acid (Legal). It also gives a cherry-red colour to a pine shaving moistened with HCl.

Scatole, β -Methyl Indole, C_9H_7N

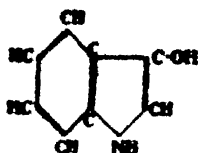


This is present in faeces. It is sometimes excreted in the urine as scatoxyl sulphuric acid. It crystallizes in shining colourless plates, m.p. 95° , and has a strong faecal odour. It is volatile in steam. It is less soluble in water than indole but dissolves easily in alcohol and ether. With conc. HCl it gives a violet colour. On warming with conc.

H_2SO_4 it gives a purple-red colour. With an alcoholic solution of p-dimethylamino-benzaldehyde and conc. HCl it gives a bluish violet colour.

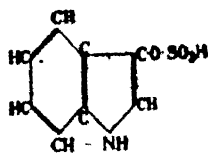
Indoxyl, β -Hydroxy Indole, $\text{C}_8\text{H}_7\text{ON}$.

This occurs in human urine as the potassium salt of indoxyl-sulphuric acid, known as *urinary indican*, $\text{C}_8\text{H}_7\text{NSO}_4$. Indoxyl is



Indoxyl

formed by the decomposition of tryptophane, and the amount of indican present in the urine gives some idea of the extent of bacterial decomposition in the intestine. It is easily converted into indigo blue

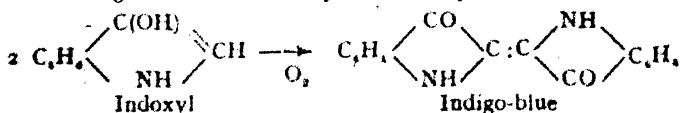


Urinary indican

and can thus be detected in the urine. The urine is mixed with the same volume of conc. HCl and a little chloroform poured in; a dilute solution of bleaching powder is added drop by drop or a trace of FeCl_3 or conc. HNO_3 , and the mixture is vigorously shaken for a few seconds and allowed to stand. The indigo blue formed dissolves in the chloroform giving it a blue colour.

Indigo Blue, $\text{C}_{16}\text{H}_8\text{N}_2\text{O}_2$.

This occurs in the indigo plant as a *glucoside indican* $\text{C}_{16}\text{H}_{17}\text{O}_5\text{N}$ which should not be confused with the *urinary indican* described above although unfortunately they both bear the same name. When the indigo plant is steeped in water, the specific enzyme present in the plant hydrolyzes the glucoside into *indoxyl* and glucose. On agitation, the oxygen of the air converts the indoxyl into the insoluble indigo-blue, which is separated and pressed into cakes.



For dyeing purposes, the insoluble indigo-blue is converted by reducing agents into the soluble *indigo-white* $\text{C}_{16}\text{H}_{10}\text{O}_2\text{N}_2$. The fabric is soaked in indigo-white solution and exposed to air, when indigo is deposited in the fabric. Indigo-blue is now manufactured cheaply by synthetic methods.

CHAPTER XXVII

ALKALOIDS

Nature and Occurrence

The term *alkaloid*, as usually applied, means a complex nitrogenous substance, obtained from plants, having basic properties and whose nitrogen atom generally forms part of a ring. They generally possess some physiological action, and from the medical point of view they are very important. Many of them being very potent poisons, play an important role in clinical toxicology and in crimes.

The alkaloids occur in plants as *salts* in combination with some common *organic acids* such as malic acid, oxalic acid, succinic acid, tannic acid, etc., or sometimes with special acids, *e.g.*, morphine with meconic acid, quinine with quinic acid, and so on. The actual amount of an alkaloid is small but by cultivation and selection the yield can usually be improved.

Classification of the Alkaloids

This is generally based on their nuclear structures. The following are some of the typical alkaloids classified according to the *nuclear structure*:

<i>Nucleus</i>			<i>Alkaloid</i>
I.	Pyridine	Nicotine, anabesine, coniine, lobeline, etc.
II.	Quinoline	Quinine, cinchonidine, etc.
III.	Indole	Strychnine, brucine, ergo- toxine, harmine, yohim- bine, etc.
IV.	Isoquinoline	Morphine, emetine, hydra- stine, corydaline, tubo- curarine, etc.
V.	Glyoxaline	Pilocarpine, etc.

<i>Nucleus</i>	<i>Alkaloid</i>
VI. Tropane	Atropine, hyoscyamine, cocaine, dioscorine, etc.
VII. Lupinane	Lupinine, sparteine, cytisine, etc.
VIII. Phenanthridine ...	Lycorine, tazettine, etc.
IX. Alkaloidal Amines ..	Ephedrine, colchicine, muscarine, etc.
X. Undetermined ...	Aconitine, alstonine, conesine, ajmaline, solanine, etc.

General Properties and Reactions of Alkaloids

As a rule they contain the four elements C, H, N and O, but a few are known (such as, nicotine, coniine, etc.) which do not contain oxygen. A few alkaloids (such as nicotine and coniine) are liquids and volatile in steam, but the majority are crystalline solids which are non-volatile. A very few alkaloids, such as berberine, serpentine, etc., are coloured but the vast majority are colourless. The free bases are, as a rule, insoluble in water but they dissolve in neutral organic solvents such as chloroform, ether, benzene, alcohol, etc. The salts, on the other hand, are very soluble in water and fairly soluble in hot alcohol but almost insoluble in solvents like ether, chloroform or benzene, and they crystallize well. The free bases can thus be precipitated from their aqueous solutions by alkalis, and extracted by means of ether, chloroform, benzene, amyl alcohol, etc. The naturally occurring alkaloids are all optically active, usually lævorotatory. The salts may sometimes possess an optical activity opposite to those of the free bases, *e.g.*, nicotine or aconitine. They have generally a bitter taste and possess marked physiological or toxic properties.

They are usually tertiary bases and have an alkaline reaction in solution. The salts with strong acid may have an acid reaction owing to partial hydrolysis. The presence of an alkaloid may be suspected if a precipitate is obtained with any of the following reagents, usually referred to as *alkaloidal reagents*. It should, however, be carefully noted that these so-called alkaloidal reagents also give precipitates with proteins, amines, etc. The importance of this lies in

the fact that in the chemical examination of medico-legal cases decomposition products of proteins of animal tissues are encountered that give reactions similar to alkaloids. Hence they are known as animal alkaloids, e.g., putrescine, cadaverine, etc., which are collectively known as *ptomaines* (Gk. *ptoma*—corpse).

Alkaloidal Reagents.—(a) A solution of mercuric iodide in potassium iodide, known as *Mayer's reagent*, gives an amorphous white precipitate. (a₁) A solution (100 c.c.) of mercuric chloride (1.35 g) and KI (5.0 g), which is double the strength of Mayer's reagent, is known as *Tanret's reagent* and is used for the estimation of minute amounts of quinine in tissues. (b) A solution of iodine in potassium iodide, known as *Wagner's reagent*, gives an amorphous brownish precipitate. (c) A solution of bismuth potassium iodide, known as *Dragendorff's* or *Kraut's reagent*, gives an orange-red precipitate. (d) A solution of phosphotungstic acid, known as *Scheibler's reagent*, gives a white amorphous precipitate. (e) A cold saturated aqueous solution of *picric acid* gives a crystalline yellow precipitate. (f) Dilute aqueous solutions of *platinic chloride* or *gold chloride* give crystalline yellow compounds which have characteristic melting points and are, therefore, used both for identification as well as for the determination of the molecular weights. For further details see Toxicology (pp. 479—481).

General Principles for the Isolation of Alkaloids.

The alkaloids occur in *plants* as salts of organic acids, and usually two or more alkaloids occur together in the same plant. The method of isolation of the *total alkaloids* and the separation of the individual members depend largely on their properties and the process may vary a good deal. It is, however, desirable to remember a general method of extraction of the total alkaloids from a plant which is partially applicable to most alkaloids.

For the isolation of alkaloids and other toxic organic compounds from *animal organs* such as liver, stomach, kidney, &c., generally called viscera, a modification of the original *Stas-Otto Process* is used in medico-legal work, and the general principle of this process is also described later. See Toxicology (pp. 427—431).

(1) *From Plants*.—The air-dried material is coarsely powdered and extracted in a large glass percolator, capacity about 5 litres (see Fig. 40) at room temperature with rectified spirit for 24 hours. The extract is drawn off and the drug soaked in fresh rectified spirit, and the process is continued until no appreciable amount of alkaloid is extracted. The alcohol is at first recovered by distillation under ordinary pressure and finally under reduced pressure. The residue is taken up with a dilute solution (about one per cent) of HCl until all the alkaloid is extracted. The acid aqueous extract is filtered, taken in a separating funnel and made alkaline with a solution of sodium carbonate or ammonia. The bases set free are extracted by shaking several times with an organic solvent, usually chloroform. The chloroform solution is dried with anhydrous

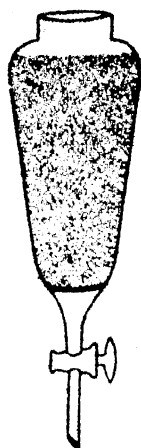


Fig. 40

potassium carbonate, filtered, and the solvent recovered by distillation. The residue, which now contains the 'total alkaloids', is dried and weighed. This residue may contain two or more alkaloids and the individual members are separated and purified by special methods depending upon the properties of the components.

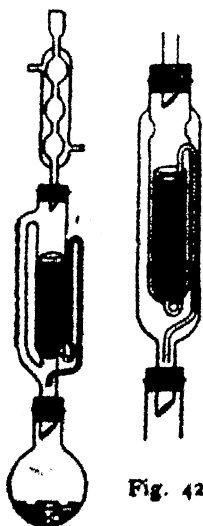


Fig. 42

Fig. 41

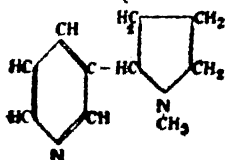
N.B. For a quick and complete extraction of a plant material on a small scale with an organic solvent such as petroleum ether, ether, chloroform, alcohol, etc., as may be desirable for a qualitative or quantitative investigation of a drug the usual *Soxhlet apparatus* (See Fig. 41) is employed. An improved pattern of the apparatus in which the extraction takes place with the hot solvent, is also shown here (See Fig. 42).

(2) *From Animal Organs*.—See the Stas-Otto method of extraction (pp. 427—431).

I. ALKALOIDS WITH A PYRIDINE NUCLEUS

Nicotine, β -Pyridyl- α -N-methyl-pyrrolidine, $C_{10}H_{14}N_2$.

Occurrence.—Nicotine occurs along with several other alkaloids (such as, nicotimine, anabesine, nicotyrine, etc.) in the *Nicotiana* species of plants of which tobacco, *Nicotiana tabacum* Linn., is the best known. The alkaloid is present in tobacco leaves as a salt of malic acid or citric acid. The total alkaloids in dried tobacco leaves may go up to 6.0 per cent. of which nicotine usually forms about 97 per cent. For other details, see Toxicology, pp. 478—483.



Preparation from Tobacco Leaves.—The dry leaves are finely powdered and then exhausted with water or very dilute acid. After filtration and concentration the extract is made alkaline with a slight excess of caustic soda and subjected to steam distillation, when the nicotine distils over. The distillate is neutralized with oxalic acid and concentrated. When cool, ether is added and the solution made alkaline; it is then well shaken to ensure extraction of the alkaloids by the ether. The ethereal solution is separated and the ether evaporated off. The residual liquid is dried in a current of hydrogen and nicotine is obtained pure by fractional distillation under reduced pressure.

Properties.—Nicotine is a colourless oily liquid with a peculiar odour and a burning unpleasant taste; sp. gr. 1.009 at 20°. It boils at about 247° with partial decomposition, but distils unchanged under reduced pressure. On keeping it turns brown. It is volatile with steam. It is readily soluble in water, alcohol, ether, chloroform and petroleum ether. The sp. rotation of the pure base, $[\alpha]_D^{20} = -168.5^\circ$. The sp. rotation of the hydrochloride $B.HCl$, $[\alpha]_D = +102.2^\circ$ (in water) and that of the sulphate, $B_2.H_2SO_4$, $[\alpha]_D = +84.8^\circ$. The picrate crystallizes in prisms, m.p. 224°.

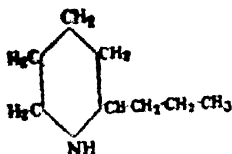
Tobacco is largely used for smoking, chewing and as snuff. Its effects on health have been the subject of many investigations. The alkaloid is certainly very poisonous and

most of us have probably experienced its effects in a mild form on our first attempts to use it. There may be a feeling of nausea, sweating and a sense of collapse. Habituation rapidly develops, but over-smoking is liable to be followed by pains in the heart region. Crude nicotine frequently adulterated with pyridine is used as an agricultural insecticide.

For tests see pp. 481—482.

Coniine. *α*-Propyl-piperidine, $C_8H_{17}N$

Occurrence.—This alkaloid is found along with other alkaloids (such as methylconiine, coniceine, conhydrine, etc.) in hemlock, *Conium maculatum* Linn. Historically, it may be of interest to note that the juice of the hemlock was used by the Greeks as a state or judicial poison for criminals, and Socrates met his death by means of this poison. The fruit, which is used in medicine, contains from 0.7 to 1.0 per cent of total alkaloids, of which coniine may constitute about 10 per cent.



Preparation.—The fruit of the plant is crushed, made alkaline with sodium carbonate and distilled in steam. The distillate obtained is neutralized with HCl and evaporated to dryness. The residue is extracted with dry alcohol which dissolves the salts of the alkaloids. The alcohol is removed and the salts dissolved in water. The bases are liberated by caustic soda and extracted with ether. The ether is removed at a low temperature and coniine is separated from the other alkaloids by fractional distillation in a current of hydrogen.

Properties.—d-Coniine, the naturally occurring alkaloid, is a colourless, strongly alkaline liquid with a penetrating odour and burning taste, b.p. 167° ; sp. gr. 0.843 at 19° . It is very slightly soluble in water but dissolves easily in alcohol and ether. The base has a sp. rotation $[\alpha]_D^{19} = +15.7^{\circ}$ (pure liquid). The hydrobromide B.HBr, which crystallizes in needles and melts at 211° , is used as a sedative. The picrate, which crystallizes from hot water in yellow needles, melts at 75° .

II. ALKALOIDS WITH A QUINOLINE NUCLEUS

Cinchona Alkaloids.—The cinchona tree, which originally came from South America, is now grown widely in Java, which supplies nearly 90 per cent of the world supply of quinine. It is also cultivated at Mungpoo in the Darjeeling district, in the Nilgiri Hills in Southern India, and in Ceylon. There are several species of the cinchona tree, such as *Chinchona officinalis* Hook, *C. succirubra* Pav., *C. calisaya* Wedd., *C. ledgeriana* Moens, etc. In Java, the bark of *C. succirubra* has been found by cultivation to yield nearly 10 per cent of total alkaloids with a preponderance of quinine and cinchonine. The species growing in Mungpoo is *C. ledgeriana* Moens and the hybrid between *C. ledgeriana* and *C. succirubra*, and shows a content of 5 to 7 per cent of total alkaloids in the bark.

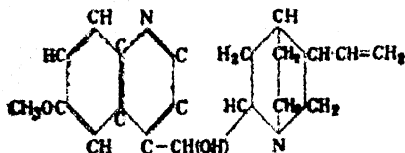
There are several alkaloids in cinchona bark and they may be divided into *crystallizable* and *amorphous* alkaloids. Among the crystallizable alkaloids, the more important ones are quinine, quinidine, cinchonine and cinchonidine.

The term **cinchona febrifuge** is applied to a mixture of the total alkaloids obtained from cinchona bark (*C. ledgeriana*, *C. succirubra*, and other suitable species of cinchona) after removing the bulk of quinine. This forms a cheap antimalarial drug and should contain not less than 7 per cent of quinine and not less than 50 per cent of total crystallizable cinchona alkaloids. A better standardized preparation, known as **totaquina**, has been suggested by the League of Nations. Totaquina (totaquine), as defined in B.P. 1948, is a mixture of the alkaloids from the bark of *Cinchona succirubra* Pav., *C. robusta* Howard, and other suitable species of *Cinchona* containing not less than 70 per cent. of crystallizable cinchona alkaloids, of which not less than one-fifth is quinine.

Quinine, $C_{20}H_{24}O_2N_2$

Preparation.—Quinine and other alkaloids are present in the cinchona bark chiefly as salts of quinic acid and of cinchotannic acid. The bark is dried and powdered. It is then made into a paste with fresh milk of lime in order to liberate the bases from their salts, and the paste dried. The powder is then extracted hot with a fairly high boiling

fraction of petroleum, and the alkaloids are removed from the petroleum extract



by shaking with successive quantities of dilute sulphuric acid. The acid extract is neutralized with caustic soda

and concentrated under reduced pressure. On cooling, crystals of *quinine sulphate*, which is less soluble than the sulphates of the other alkaloids, separate out. It is filtered off and purified by recrystallization from water with the help of animal charcoal. The filtrate from the precipitated quinine sulphate is utilized in preparing the other alkaloids or for the preparation of *totaquina* or *cinchona febrifuge*. For the preparation of *quinine* (base), the pure sulphate is dissolved in hot water and precipitated by caustic soda. The base is washed with water, dried, and recrystallized from alcohol. For the preparation of the *bihydrochloride*, used for injections, the acid sulphate is treated with the calculated amount of BaCl₂, avoiding any excess of the latter:



Properties, Uses and Tests.—Quinine (base) crystallizes from alcohol in colourless needles, m.p. 173.5° (dry). When obtained by precipitation from a salt by means of an alkali, it is found as an amorphous, colourless powder. It is very sparingly soluble in water (1 in 1750 at 25°) but is easily soluble in alcohol, ether, and chloroform. Sp. rotation $[\alpha]_D^{15} = -158^\circ$ (in 99 per cent alcohol). It is a diacid base and forms *neutral* and *acid* salts. The base and the salts are all very bitter in taste. Quinine sulphate, $B.H_2SO_4.7\frac{1}{2}H_2O$, is the *neutral sulphate*, and crystallizes from boiling water in shining needles; these effloresce on exposure to air or when kept over conc. H_2SO_4 and form $B.H_2SO_4.2H_2O$, m.p. 205°. The salt $B.H_2SO_4.7\frac{1}{2}H_2O$ is soluble with difficulty in water (1 in 720 at 25°), but is more soluble in alcohol or better in a mixture of 2 parts of chloroform and one part of alcohol. It shows the sp. rotation, $[\alpha]_D^{15} = -166.36^\circ$ (alcohol). The *acid sulphate* $B.H_2SO_4.7H_2O$ forms colourless ortho-rhombic crystals, m.p. 160°, sp. rotation $[\alpha]_D = -216^\circ$ (dry salt in water).

It is easily soluble in water and alcohol. Quinine *hydrochloride* $B.HCl.2H_2O$ crystallizes in needles and is easily soluble in water, alcohol and chloroform; sp. rotation $[\alpha]_D = -155.8^\circ$ (water). The aqueous solution is neutral to litmus. Quinine *bihydrochloride* or acid hydrochloride $B.2HCl$ crystallizes in colourless needles, and is very soluble in cold water (1 in 0.75). The hydrochlorides and sulphates are very largely used in medicine, especially in the treatment of malaria, the bihydrochloride being used for injections.

Aristochin, the carbonic ester of quinine, $(C_{20}H_{22}O_2N_2)_2CO$, is a tasteless derivative used for infants. It is a white powder, m.p. 189° ; it is almost insoluble in water but is easily soluble in alcohol. It gives the thalleioquin reaction, given by quinine (see below).

Euquinine, Quinine ethyl carbonate, $C_{20}H_{22}O_2N_2.COO.C_2H_5$, prepared by the action of ethyl chlorocarbonate on quinine; a tasteless, colourless, crystalline powder, m.p. 95° , used for children; slightly soluble in water easily soluble in alcohol and dilute acids. It gives the thalleioquin reaction.

Tests for Quinine: (1) a solution of quinine in dilute sulphuric acid exhibits a bluish fluorescence. (2) *Thalleioquin reaction:* If bromine- or chlorine- water is added drop by drop to a slightly acid solution of a quinine salt until the bromine or chlorine is present in very slight excess and then an excess of dilute ammonia is added, an emerald green colour is produced. On adding to this a few drops of a freshly prepared solution of potassium ferricyanide, the colour turns red (*roseoquinine*).

Quinine is largely excreted by the kidneys and so by testing the urine it is easy to tell whether a patient is taking his quinine or not.

Quinidine, $C_{20}H_{24}O_2N_2$. This is an isomer of quinine being dextrorotatory whereas quinine is laevorotatory. It is found in small quantities in most cinchona barks, *C. calisaya* Wedd. being specially rich in this.

Preparation.—It can be prepared from the mother liquor, from which quinine sulphate has crystallized out, by precipitating all the alkaloids with caustic soda. The precipitate is extracted with ether which dissolves out quinidine and cinchonidine. The residue from ether is dissolved in dilute sulphuric acid, neutralized with an alkali and treated with a solution of sodium potassium tartrate which precipitates the cinchonidine. From the filtrate, quinidine is precipitated as base, washed, dried and crystallized from alcohol.

Properties.—Quinidine crystallizes from alcohol in colourless prisms. When anhydrous it melts at 173.5° and has the sp. rotation $[\alpha]_D = +274.7^\circ$ (mixture of 1 vol. alcohol + 2 vols. $CHCl_3$). It is scarcely soluble in water, sparingly soluble in chloroform and petro-

leum ether but is easily soluble in ether and alcohol. It is a diacid base and forms two series of salts. It gives the thalleioquin reaction and shows bluish fluorescence in dilute sulphuric acid. It is now widely used in the treatment of a certain type of heart disease.

Cinchonine, $C_{11}H_{21}ON_2$

Occurrence and Preparation.—This alkaloid is present in small quantities in all the varieties of cinchona plant. It differs from quinine or quinidine in having the methoxyl group (CH_3O) of the quinoline ring replaced by hydrogen.

It may be prepared from the mother liquor, from which quinine sulphate has crystallized out, by precipitating all the alkaloids with caustic soda. The precipitate is extracted with ether to remove the quinidine and cinchonidine. The residue is dissolved in the minimum amount of boiling alcohol. On cooling, cinchonine crystallizes out. It is further purified by recrystallizing the sulphate from hot water.

Properties.—Cinchonine crystallizes in colourless prisms, m.p. 264° ; sp. rotation $[\alpha]_D^{17} = +229^\circ$ (absolute alcohol). It is scarcely soluble in water but dissolves in alcohol and ether. It is a diacid base and forms two series of salts. It does not give the thalleioquin reaction and does not show fluorescence in dilute sulphuric acid.

Cinchonidine, $C_{11}H_{21}ON_2$

Occurrence and Preparation.—This is an isomer of cinchonine, and occurs in small quantities in all the cinchona plants, *C. succirubra* Pav. being richer in this alkaloid. It is prepared from the precipitate obtained with sodium potassium tartrate (see quinidine) by dissolving it in dilute acid and precipitating the base with excess of ammonia. The precipitate is washed, dried and recrystallized from alcohol.

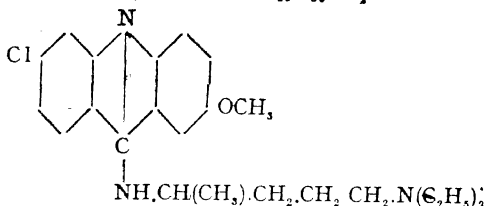
Properties.—Cinchonidine crystallizes from alcohol in colourless prisms, m.p. 204.5° ; sp. rotation $[\alpha]_D = -111^\circ$ (alcohol). It is scarcely soluble in water, soluble in alcohol and ether. It does not give the thalleioquin reaction and does not fluoresce in dilute sulphuric acid. It is a diacid base and forms two series of salts.

SOME SYNTHETIC ANTIMALARIALS

The use of quinine in the treatment of malaria possesses certain disadvantages. Some susceptible individuals show symptoms of cinchonism and it cannot be safely given to pregnant women or in cases of black-water fever. The desire to discover a compound which would be superior in action to quinine as well as to solve the problem of the shortage of quinine during a prolonged war led to the synthesis of numerous compounds, and three of these which

have stood the test of experiments in malaria are mentioned here.

Mepacrine, Atebrin, 2-Chloro-5-(*w*-diethylamino- α -methyl butylamino)-7-methoxy acridine, $C_{23}H_{30}ON_2Cl$

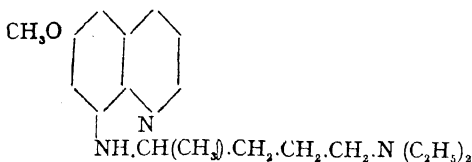


The base, which is shown in the diagram, is prepared by the action of *w*-diethylamino- α -methyl butyl amine upon 2:5-dichloro-7-methoxy acridine. *Mepacrine dihydrochloride*, $C_{23}H_{30}ON_2Cl \cdot 2HCl \cdot 2H_2O$ (M.W. 508.7), which is used in medicine, is a bright yellow crystalline powder with a bitter taste. It is soluble in about 40 parts of water. *Mepacrine methansulphonate*, $C_{23}H_{30}ON_2Cl \cdot 2CH_3SO_3H \cdot H_2O$ (M.W. 610.2) is a bright yellow crystalline solid with a bitter taste soluble in about 3 parts of water and in about 36 parts of 95 per cent alcohol; it is used for injection.

Mepacrine can be estimated by making the solution of the salt alkaline with dilute caustic soda, extracting the base with chloroform, removing the solvent and drying at 100° .

This drug is effective against the asexual forms of Quartan, B.T., and M.T. parasites, the latter being the most vulnerable to its action; it has no action on the sexual forms of the parasites.

Pamaquin, Plasmoquine, 6-Methoxy-8-(*w*-diethylamino- α -methyl butyl)-aminoquinoline salt of 2:2'-dihydroxy-1:1'-dinaphthylmethane-3:3'-dicarboxylic acid, $C_{42}H_{45}O_7N_3$ (M.W. 703.4)



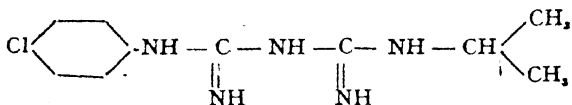
The base, which is shown in the diagram, is prepared by the action of 2-chloro-5-diethylamino pentane upon 6-methoxy-8-aminoquinoline. Pamaquin (the above salt) is a yellow to orange yellow powder with a bitter taste, almost insoluble in water, but soluble (1 in 10) in acetone containing 5 per cent of water.

The base can be estimated by making a solution of the salt alkaline with dilute caustic soda, extracting the base with benzene removing the solvent and drying it at 100° . The salt should contain 43 to 45 per cent of the base.

Pamaquin has a very definite lethal action on the gametocytes (sexual forms) of M.T. parasites, but in non-toxic doses it has no appreciable effect on the asexual forms of M.T. or the sexual and asexual forms of P.T. and Quartan parasites.

Paludrine

This is the latest antimalarial drug discovered by British scientists. It is a guanidine derivative of p-chloro-benzene and is stated to have the following constitutional formula.



It is a white powder with a bitter taste, soluble in about 50 parts of water.

It is stated to act not only on all the three forms of malaria parasites in the blood but also to have effect on those forms of the parasites which reside within the solid tissues (reticulo-endothelial system) during the incubation period of the disease.

Some of the more recent and useful synthetic antimalarials are mentioned below:

1. **Chloroquine**, *Resochin*, *Aralen*, S.N. 7618; this is 7-chloro-4-(4-diethylamino-1-methyl butyl amino) quinoline, and is used as diphosphate; its properties are similar to those of mepacrine, but is stated to possess about 3 times its activity and does not colour the skin.

2. **Pentaquine** or S.N. 13276; this is 8-(5-isopropyl amino amylamino)-6-methoxy quinoline, and is used as monophosphate.

3. **Camouquin** or *Cam-AQI*; this is 4-(3'-diethylamino methyl-4'-hydroxyanilino)-7-chloroquinoline, and is used as dihydrochloride dihydrate; yellow crystalline powder; one gram dissolves in 5 c.c. of water at 25°C .

III. ALKALOIDS WITH INDOLE NUCLEUS

Strychnine, $\text{C}_{21}\text{H}_{22}\text{O}_2\text{N}_2$

Occurrence.—Strychnine and brucine occur together in *Nux Vomica* seeds (*Strychnos nux vomica* Linn.) and in

Ignatia beans (*Strychnos ignatii* Berg). Both of them contain 2 to 3 per cent of total alkaloids, and in *Nux Vomica* seeds strychnine forms about half the total alkaloids whereas in *Ignatia* beans it forms about two-thirds of the total alkaloids.

Preparation.—The powdered dry seeds of *Nux Vomica* are intimately mixed with slaked lime and a little water. The mass is dried, powdered and extracted with hot chloroform. The alkaloids are removed from the chloroform extract by agitation with dilute sulphuric acid, and the bases precipitated from the acid solution with excess of ammonia, filtered and dried. On treating the mixture of alkaloids with 25 per cent alcohol, the brucine goes into solution while most of the strychnine remains undissolved, and the latter is purified by recrystallization from alcohol.

Properties.—Strychnine crystallizes in colourless prisms, m.p. 268° . The base is very slightly soluble in water or ether, but is soluble in 90 per cent alcohol and in benzene; it is easily soluble in chloroform. The sp. rotation of the base $[\alpha]_D = -139.3^{\circ}$ (in chloroform). The base is monoacidic and forms salts with acids of which the nitrate $B.HNO_3$, sulphate $B_2.H_2SO_4.5H_2O$ and hydrochloride $B.HCl. 2H_2O$ are used in very small doses in medicine as a tonic or heart stimulant. Strychnine is highly toxic and is used as a vermin killer and in killing stray dogs in the streets of Calcutta.

Tests.—For tests see Toxicology (pp. 494-495).

Brucine, $C_{23}H_{26}O_4N_2$

Preparation.—As mentioned under strychnine, brucine is found in solution when the mixed alkaloids from *Nux vomica* seeds are extracted with 25 per cent alcohol. The solution is concentrated and the bases precipitated as neutral oxalate. The oxalates are dried and extracted with dry alcohol in order to remove the strychnine oxalate. The brucine oxalate is dissolved in hot water, decolorized with animal charcoal, filtered, and the base precipitated by ammonia. The base is converted into sulphate which is purified by recrystallization from water. The base liberated from the pure sulphate may be further purified by recrystallization from aqueous alcohol.

Properties and Tests.—The alkaloid crystallizes from dilute alcohol in colourless prisms, $B. 4H_2O$, m.p. 105° ; m.p. of anhydrous base 178° ; sp. rotation $[\alpha]_D = -119^{\circ}$ to -127° (chloroform). It is slightly soluble in cold water but more so in hot water. It is very

soluble in alcohol, chloroform and amyl alcohol, but less so in ether. It is a monacid base, forming salts like $B \cdot HCl$, $B \cdot H_2SO_4 \cdot 7H_2O$, etc.

Tests.—For tests see Chapter 34.

Ergotoxine, $C_{33}H_{41}O_6N_5$.

Occurrence.—This alkaloid occurs in *ergot* which consists of the mycelia of the *Claviceps purpurea* found to grow on cereals, best on rye. Ergot contains several alkaloids, such as ergotoxine, ergotinine, ergotamine, ergometrine, etc., and a good sample of ergot should contain not less than 0.05 per cent of total alkaloids, calculated as ergotoxine.

Preparation.—Powdered ergot is extracted with alcohol until exhausted and the alcohol is recovered by distillation. The residue is washed with petroleum ether to remove oily matter and extracted with ethyl acetate. The ethyl acetate solution is extracted with a solution of citric acid until all the alkaloids are removed. Sodium bromide is added to the citric acid solution to precipitate ergotoxine and ergotinine as hydrobromides. These hydrobromides are dissolved in water, made alkaline with caustic soda and extracted with ether to remove the ergotinine. The alkaline solution is neutralized and again made alkaline with sodium carbonate and extracted with ether which dissolves out the ergotoxine. The residue obtained from the ether is dissolved in alcohol and converted into the phosphate. The ergotoxine phosphate is purified by recrystallization from alcohol.

Properties.—Ergotoxine phosphate $B \cdot H_2PO_4 \cdot H_2O$ crystallizes in needles m.p. 186° it is soluble in water and alcohol. Ergotoxine shows all the characteristic physiological action of ergot and is used as a standard for the assay of ergot preparations. Ergot is mainly used in medicine for producing contraction of the uterus after delivery.

IV. ALKALOIDS WITH AN ISOQUINOLINE NUCLEUS

Opium Alkaloids

Opium.—This is the air-dried latex or milky exudation obtained by incising the unripe capsules of the opium poppy (*Papaver somniferum* Linn.), which is grown in India,

China, Persia and Asia Minor (Smyrna). Opium, as usually found, is a dark tenacious substance and comes to the market in the form of balls about the size of a coconut. The substance itself has a peculiar aroma which is very characteristic. See opium in Toxicology, pp. 483—490.

Opium is used largely by Hakims and Kavirajes and is also used in Western medicine but now-a-days its use has been largely displaced in scientific medicine by its alkaloids, chiefly morphine. Opium is largely used in medicine as a sedative and hypnotic drug. Apart from its legitimate use, it is frequently employed for smoking as *chandu* and *gooli* and also for eating.

The two most important *alkaloids of opium* are morphine and codeine, but about 23 other alkaloids (narcotine, thebaine, papaverine, protopine, etc.) have been isolated from opium. The amount of total alkaloids in Indian opium may go up to 40 per cent, and Government opium contains about 10 per cent of morphine and about 1.4 per cent of codeine. The alkaloids found in opium occur in combination with *meconic acid*. Besides the alkaloids there are *proteins*, *waxes*, *gums*, *resins*, *sugars*, *organic acids*, and some other constituents. The presence of the alkaloid porphyroxine is believed to be the characteristic feature of Indian opium.

Morphine, $C_{17}H_{19}O_3N$.

Preparation.—This is prepared from opium by extracting it with hot water. The hot water extract is treated with chalk and concentrated calcium chloride solution is added followed by a little water. A precipitate of resin and calcium meconate forms, which is filtered off and the filtrate is concentrated, when morphine and codeine hydrochlorides crystallize out. To separate morphine from codeine, the hydrochlorides are again dissolved in water and excess of ammonia added which precipitates most of the morphine with a little of the codeine. The morphine is purified by recrystallization from hot alcohol.

Properties.—Morphine crystallizes from alcohol in colourless prisms with one molecule of water of crystallization, m.p. 254° (with decomposition); sp. rotation

$[\alpha]_D^{23} = -130.9^\circ$ (methy alcohol). It is bitter in taste and is very sparingly soluble in water, ether, chloroform or benzene. It is fairly soluble in hot alcohol, in amyl alcohol and in ethyl acetate. Morphine has got two hydroxyl groups, one *phenolic* and the other *alcoholic*. Owing to the phenolic hydroxyl group, it is easily soluble in lime water and in alkali hydroxides, and gives the colour reaction with ferric chloride. It reduces salts of gold and silver, permanganate, iodic and periodic acids. When heated with strong alkalis, HCl or zinc chloride, it loses a molecule of water and is converted into *apomorphine* $C_{17}H_{17}O_2N$, a powerful emetic drug.

Morphine is a monacid base. The hydrochloride, $B.HCl.3H_2O$, used in medicine, crystallizes in colourless needles; sp. rotation $[\alpha]_D^{15} = -100.67^\circ + 1.14c$, where c =conc. in water. The sulphate $B_2.H_2SO_4.5H_2O$ crystallizes in colourless silky needles; sp. rotation $[\alpha]_D^{15} = -100.47^\circ + 0.96c$, where c =conc. in water. It is a very powerful habit-forming drug (morphomaniac).

Tests.—For tests see pp. 487—489.

Heroin, Diacetylmorphine hydrochloride $C_{17}H_{17}NO_4(CO.CH_3)_2.HCl$.—This is prepared by heating morphine with acetic anhydride, thus replacing the two hydrogen atoms of the two hydroxyl groups of morphine with two acetyl groups. The hydrochloride, m.p. 232° , is soluble in water and alcohol. It is used in medicine as a sedative and is stated to cause less mental depression than morphine.

Codeine, $C_{18}H_{21}O_3N$.

Occurrence and Preparation.—This alkaloid may occur in opium to the extent of 0.1 to 3.0 per cent. It is a methyl ether of morphine, the hydrogen atom of the phenolic hydroxyl group being replaced by methyl group. It is, therefore, often synthesized from morphine by methylation. It is prepared from opium by treating the mother liquor, from which morphine (see this) has been precipitated by ammonia, with caustic potash when the codeine is precipitated. The codeine is then purified by recrystallization from ether or benzene.

Properties and Uses.—Codeine crystallizes from water in colourless prisms with one molecule of water of crystallization, m.p. 155° (anhydrous). It is fairly soluble in hot water, but more so in alcohol, chloroform, ether and benzene;

sp. rotation $[\alpha]_D = -111.5^\circ$ (chloroform). It is sparingly soluble in aqueous solutions of alkali hydroxides, and is a strong monacid base. The hydrochloride $B \cdot HCl \cdot 2H_2O$ crystallizes in needles; sp. rotation $[\alpha]_D^{22} = -108.2^\circ$ (water). The sulphate $B_2 \cdot H_2SO_4 \cdot 5H_2O$, crystallizes in colourless prisms, m.p. 278° ; sp. rotation $[\alpha]_D^{15} = -101.2^\circ$ (water). The base as well as the sulphate and phosphate are used as hypnotics and sedatives.

For tests see p. 492.

Emetine, $C_{29}H_{46}O_4N_2$

Occurrence.—Emetine is found in the roots of *Psychotria ipecacuanha* Mull.—Arg. (*Ipecac*) found in Brazil. The plant is also cultivated in the Federated Malay States and has more recently been introduced into India and is growing at Mungpoo in the Darjeeling district. Besides emetine, the most important alkaloid, ipecac contains smaller quantities of cephaeline, psychotrine, emetamine, etc. Brazilian roots may contain about 2.5 per cent of total alkaloids of which about 70 per cent may be emetine. The ipecac roots from Mungpoo have been found to contain about 2.6 per cent of total alkaloids with 1.34 per cent of emetine. The introduction of emetine in Indian medicine has revolutionized the treatment of the comparatively common complaint amoebic dysentery and hepatitis which in the pre-emetine days were so often associated with serious complications.

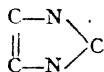
Preparation.—The powdered roots are extracted with alcohol, the extract is concentrated under reduced pressure, diluted with water and washed with ether to remove oily and resinous impurities. The aqueous liquid is made alkaline with sodium carbonate or ammonia, and the alkaloids extracted with ether. The ethereal solution is washed with dilute caustic soda to remove the phenolic alkaloids (cephaeline and psychotrine) and the emetine finally taken up with dilute hydrochloric acid. It is further purified by recrystallizing the hydrochloride and liberating the base from the pure hydrochloride with ammonia.

Properties, Use and tests.—Emetine forms a white amorphous powder, m.p. 74° ; sp. rotation $[\alpha]_D = -50^\circ$ (chloroform). It is easily soluble in ether, alcohol and

chloroform but is less soluble in light petroleum and benzene. The hydrochloride $B.2HCl.7H_2O$ crystallizes from water in colourless needles, m.p. 235° — 255° (with decomposition); sp. rotation $[\alpha]_D = +53^{\circ}$ (chloroform). It is largely used in medicine in the treatment of amoebic dysentery. If given by the mouth it produces intense emesis (vomiting) and hence the name emetine. For test (B.P.), take one c.c. of conc. H_2SO_4 containing 0.005 g. of pure molybdic acid and sprinkle a small quantity of the alkaloid in powder—a bright green colour is produced.

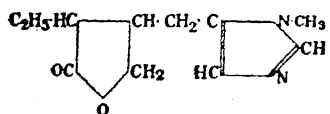
V. ALKALOIDS WITH A GLYOXALINE NUCLEUS

These are derived from the *glyoxaline* or *iminazole* ring with the following structure. Other important compounds containing this heterocyclic structure are *histidine* and *histamine*.



Pilocarpine, $C_{11}H_{16}O_2N_2$

Occurrence and Preparation.—This alkaloid occurs along with pilocarpidine in the leaves of Jaborandi, *Pilocarpus jaborandi* Holmes. It is extracted by treating the powdered leaves with acidulated alcohol. The alcohol is distilled off and the aqueous residue just neutralized with ammonia and



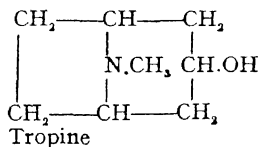
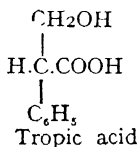
filtered to remove resins, etc. The alkaloids are liberated in the filtrate with excess of ammonia and extracted with chloroform. The residue from chloroform is dissolved in a little water and neutralized with dilute nitric acid. The pilocarpine nitrate is obtained pure by recrystallizing it from alcohol.

Properties and Use.—Pilocarpine is a colourless oil, soluble in water, alcohol and chloroform and almost insoluble in ether or petroleum ether. The sp. rotation

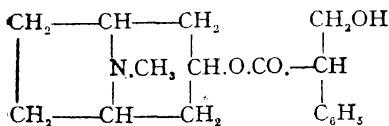
$[\alpha]_D = +100.5^\circ$ (chloroform). It is monacid base; the nitrate $B.HNO_3$ crystallizes in colourless prisms, m.p. 178° ; sp. rotation $[\alpha]_D = +82.9^\circ$ (water); the hydrochloride $B.HCl$ crystallizes in prisms m.p. 204° ; sp. rotation $[\alpha]_D = +91.74^\circ$ (water). Pilocarpine causes increased secretion by salivary, lachrymal and gastric glands and its salts are used as diaphoretics. It contracts the pupil and is used as an antidote to atropine. It promotes the growth of hair.

VI. ALKALOIDS WITH A TROPANE NUCLEUS.

The members of this group of alkaloids, often known as the *mydriatic alkaloids* on account of the property of dilating the pupil of the eye, are found in the Solanaceous plants like *Atropa belladonna* Linn. (deadly nightshade), *Datura stramonium* Linn. (thorn apple), *Hyoscyamus niger* Linn. (henbane), etc. Three members of this series are very important medically, viz., atropine (dl-hyoscyamine), l-hyoscyamine and hyoscine (scopolamine). The two former are esters of *tropic acid* and the base *tropine*, whereas hyoscine is the ester of tropic acid and the base *scopoline* (oscine).



Atropine, dl-Hyoscyamine, $C_{17}H_{23}O_3N$



Occurrence.—Atropine occurs only in traces in plants which contain l-hyoscyamine, hyoscine (scopolamine), and other alkaloids. The alkaloid is, therefore, prepared by the *racemization* of l-hyoscyamine isolated from plants such as *Datura stramonium*, *Atropa belladonna* or *Hyoscyamus niger* which are rich sources of l-hyoscyamine. Of these plants,

the last two are found to occur in India, chiefly in Kashmere. The common *Datura* found in Bengal is *D. fastuosa* (see Toxicology, Chapter 34), the seeds of which are rich in alkaloids, especially hyoscyne.

Preparation of Hyoscyamine and its Racemization to Atropine.—

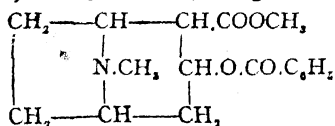
The dry roots of *Atropa belladonna* are coarsely powdered and extracted repeatedly with rectified spirit until all the alkaloids are exhausted. The alcohol is recovered under reduced pressure and the residue extracted repeatedly with one per cent HCl. The aqueous acid extract is filtered, made alkaline first with sodium carbonate solution and then with ammonia and the alkaloids extracted with chloroform. The chloroform extract is concentrated and the alkaloids extracted by shaking with dilute HCl. The acid extract is made alkaline with sodium bicarbonate and shaken 2 or 3 times with sulphuric ether, which removes the hyoscyne (scopolamine). The aqueous solution is then treated with ammonia and the precipitated hyoscyamine extracted with chloroform. The chloroform extract is dried with solid potassium carbonate, filtered and the chloroform removed. The residual crude hyoscyamine is purified by recrystallization from hot light petroleum (b.p. 80° — 120°).

To prepare atropine, purified l-hyoscyamine is racemized either by treating its solution in absolute alcohol with a dilute solution of caustic soda in absolute alcohol at room temperature for 10 or 12 hours or by heating l-hyoscyamine in chloroform solution under pressure to about 120° .

Properties and Use.—Atropine crystallizes from alcohol in colourless prisms, m.p. 118° . It is sparingly soluble in cold water but more easily soluble in hot water (1 in 87 at 80°); the aqueous solution being bitter in taste. It is easily soluble in alcohol, chloroform and ether. The sulphate B_2, H_2SO_4, H_2O is a colourless crystalline powder, m.p. 195° , and is used in medicine, especially in ophthalmic work to produce dilatation of the pupil. *Datura* is sometimes used in India for drugging as an aid to robbery (see Toxicology, p. 497).

Tests.—For tests see pp. 498-499.

Cocaine, Methyl benzoyl ecgonine, $C_{17}H_{21}O_4N$



Occurrence.—Cocaine and its associated alkaloids are obtained from Coca leaves, *Erythroxylon coca* Linn., found in Peru, Bolivia, Java and Ceylon. The amount of alkaloids in the leaves varies from 0.7 to 1.6 per cent.

Preparation.—The finely powdered dry leaves are extracted with dilute sulphuric acid, the solution treated with sodium carbonate and the precipitated alkaloids extracted with light petroleum. The alkaloids are shaken out of the petroleum with dilute sulphuric acid and reprecipitated with sodium carbonate. The base is converted into hydrochloride which is recrystallized from water and the cocaine liberated from the pure hydrochloride thus obtained is recrystallized from alcohol.

Properties.—Cocaine crystallizes from alcohol in colourless prisms, m.p. 98° . Its sp. rotation $[\alpha]_D = -15.8^{\circ}$ (chloroform). It is very sparingly soluble in water but dissolves easily in alcohol, ether, chloroform, benzene and light petroleum. It is a strong monacid base forming salts with acids. The hydrochloride B.HCl, which is commonly used in medicine, crystallizes from alcohol in colourless prisms, m.p. 201° (dry); sp. rotation $[\alpha]_D = -71.95^{\circ}$ (2 per cent aqueous solution). It is easily soluble in water but less soluble in absolute alcohol.

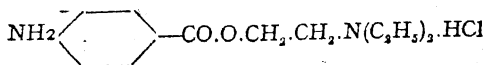
Cocaine owes its medical importance to the fact that it produces anæsthesia when applied to an absorbing surface, hence it is largely employed as a *local anæsthetic* in eye surgery, in dentistry, and in ear, nose and throat troubles. It has also a peculiar psychical action stimulating the brain and causing a feeling of well-being with the result that it is often resorted to by drug addicts with disastrous results as the habit so formed is almost impossible to break, and usually ends in complete moral and physical degeneration of the unfortunate individual. Addicts may employ subcutaneous injection of the drug or use it as a snuff; it is often alluded to as 'snow'. Cocaine addicts are quite common in India.

Tests.—For tests see pp. 504-506.

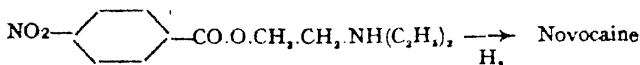
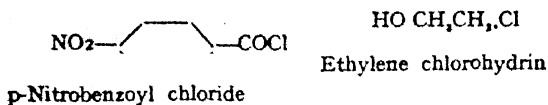
Synthetic Local Anæsthetics

Cocaine solution used for hypodermic injection becomes mouldy on keeping; it decomposes on boiling and cannot therefore be easily sterilized. Attempts have, therefore, been made to prepare synthetic local anæsthetics which would be cheaper than cocaine, more stable and less toxic, and some of these are widely used in medical practice.

Novocaine, Procaine, Diethylamino ethyl-p-aminobenzoate hydrochloride.

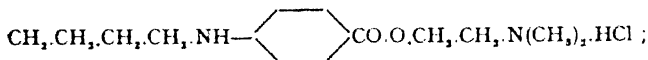


It is prepared as follows:



Novocaine is non-irritant, only one-seventh as toxic as cocaine, and possesses a powerful local anæsthetic action (see p. 506).

Pantocaine, 4-Butylamino benzoyl dimethylamino ethanol hydrochloride,



This compound is stated to possess 10 times the local anæsthetic power of novocaine.

ALKALOIDS OF UNDETERMINED CONSTITUTION

Aconite Alkaloids.—The aconite group of alkaloids are derived from the genus *Aconitum* of which several species are found in India. The aconite roots are used both in the Hindu and Mohamedan systems of medicine. Wolf's bane or Monkshood (*Aconitum napellus* Linn.) is the European species, the roots of which are imported into India, the chief alkaloid being *aconitine*. The other alkaloids found in the different species of aconite roots are *pseudaconitine*, *indaconitine*, *atisine*, etc. For further details see Toxicology (pp. 499—501).

Aconitine, $\text{C}_{34}\text{H}_{47}\text{O}_{11}\text{N}$.

Preparation.—The roots of the plant are finely ground and extracted with alcohol. The alcohol is removed under reduced pressure and the aqueous acid residue washed with ether to remove oily impurities; the aqueous solution is made slightly alkaline with sodium bicarbonate and extracted with ether which removes the aconitine, leaving the other alkaloids

(aconine, benzaconine, etc.) behind. The ethereal solution is dried with sodium sulphate and on slow evaporation of the ether the aconitine crystallizes out.

Properties.—It crystallizes in colourless prisms, m.p. 197° ; sp. rotation $[\alpha]_D = +14.6^{\circ}$ (chloroform). It is easily soluble in chloroform and benzene, less soluble in ether and almost insoluble in water. The hydrobromide $B.HBr.2\frac{1}{2}H_2O$ crystallizes in colourless tablets; sp. rotation $[\alpha]_D = -30.8^{\circ}$ (water). Aconitine is *extremely poisonous* and should be handled with caution. Aconite root is a rather commonly employed poison in India, occurring mostly in homicidal cases and also frequently accidentally.

Tests.—For tests see pp. 502-503.

CHAPTER XXVIII

ESSENTIAL OILS AND RESINS

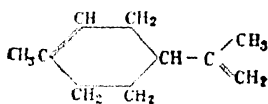
Occurrence and Uses.—The essential oils are widely distributed in nature and the characteristic odours of leaves and flowers of plants are generally due to the presence of these oils. All the natural perfumes belong to this class. Many of these perfumes are very expensive when obtained from natural sources and most of the cheap scents on the market are nowadays manufactured synthetically in the laboratory. The essential oils are used extensively in medicine as flavouring agents, carminatives, rubefacients, etc.

Physical Characters.—The essential oils differ from the fixed oils (fatty oils) by being volatile in steam. Like the fixed oils they do not produce any permanent translucent stain or grease-spot as it is called on paper, and unlike fixed oils they all possess characteristic odours. Some of them are volatile at ordinary temperatures, although the boiling points are generally high. They can all be distilled in vacuo without decomposition. Most of the essential oils are *optically active* (cf. fixed oils). They possess rather high *refractive indices*, varying from 1.46 to 1.51 at 20° ; the high refractive index (1.51) of cedar wood oil, for example,

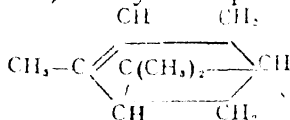
has been utilized for oil-immersion lenses in microscope work. The *specific gravity* of some are lower than water (e.g., lemon grass oil 0.899, eucalyptus oil 0.910), while others possess higher specific gravity (e.g., oil of wintergreen 1.187). The essential oils are almost insoluble in water. They dissolve freely in absolute alcohol but are precipitated by dilution with water.

Chemical Nature and Classification.—The essential oils occurring in the plants are generally mixtures of different chemical compounds. They have been classified by Parry as follows:

(1) *Terpenes*; hydrocarbons of the formula $C_{10}H_{16}$; found in lemon-grass oil, orange oil, etc.; e.g., limonene, a monocyclic terpene, and pinene, a dicyclic terpene:

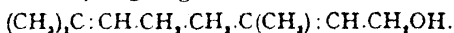


Limonene

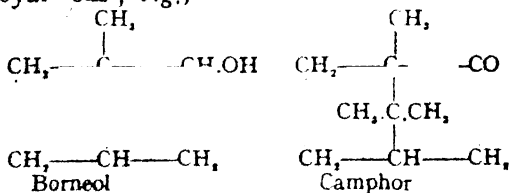
 α -Pinene

(2) *Sesquiterpenes*; hydrocarbons of the formula $C_{15}H_{24}$; found in cedar wood oil and sandal wood oil; e.g., cedrene $C_{15}H_{24}$, santalene $C_{15}H_{24}$.

(3) *Open-chain Alcohols and their corresponding Aldehydes*; found in citronella oil, lemon grass oil and Indian geranium oils; e.g., geraniol,



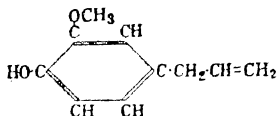
(4) *Aromatic Alcohols of the Camphor Series and their corresponding Ketones*; found in peppermint-, thuja-, and pennyroyal- oils; e.g.,



(5) *Aromatic Alcohols of the Benzene Series and their corresponding Aldehydes and Ketones*; found in bitter almond oil, caraway oil, cinnamon oil, etc.; e.g., cinnamic aldehyde, $C_6H_5.\text{CH}:\text{CH}.\text{CHO}$.

(6) *Sesquiterpene Alcohols* ; found in sandal wood oil ;
e.g., santalol $C_{15}H_{24}O$.

(7) *Phenols and their derivatives* ; found in clove oil ;
e.g., eugenol,



(8) *Esters of any of the above mentioned alcohols* ;
found in lavender oil, bergamot oil, wintergreen oil, etc. ;
e.g., methyl salicylate, $C_6H_4(OH).COOCH_3$.

(9) *Sulphur Compounds* ; e.g., diallyl disulphide,
 $C_6H_{10}S_2$, found in garlic oil ; allyl isothiocyanate, C_3H_5NCS ,
found in mustard oil, etc.

Extraction of Essential Oils

(1) *By Steam Distillation*.—The material is moistened with water and distilled in a current of steam. The oil passes over and is condensed with the steam. The oil either floats on the top or sinks to the bottom of the condensed water according to its specific gravity, and is separated by mechanical means. The portion which forms an emulsion with water is redistilled or extracted with a solvent such as ether, etc. The essential oil thus obtained is dried and distilled in high vacuo.

(2) *By a Solvent*.—The material is extracted with a low-boiling solvent such as ether or petroleum ether. This method is only used for those delicate oils which cannot be exposed to high temperature.

Turpentine is the oleo-resin that oozes out when incisions are made on the stems of various species of pine trees, such as *Pinus palustris* Mill., *P. maritima* Lam., etc. This industry is common in the hills in certain parts of India and in many other countries where pine forests abound. The crude exudate is distilled in a current of steam when the commercial oil of turpentine (10-25 per cent of the oleo-resin) distils over and the non-volatile resin, known as *rosin* or *colophony*, remains behind. The commercial oil is purified

by redistillation when the pharmacopœial *Rectified Oil of Turpentine* or *Oleum Terebinthinae* is obtained.

The rectified oil has a rather pleasant odour and the main fraction boils between 155° and 160° . It is insoluble in water. It dissolves easily in absolute alcohol, ether, petroleum ether, chloroform, and benzene, and in fixed oils. When exposed to air, it absorbs oxygen, gradually hardens and is converted into a resin. It is, therefore, widely used in making varnishes. It is also used in medicine, both for internal and external use. The oil consists mainly of *α -pinene* which is used in making artificial camphor.

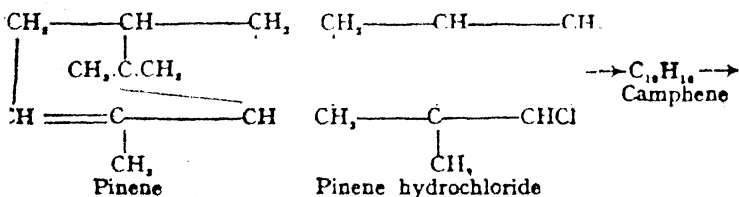
Camphor, $C_{10}H_{16}O$

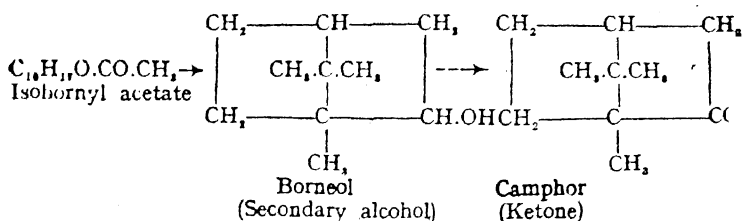
Occurrence.—Camphor occurs in the wood, twigs and leaves of the camphor tree, *Cinnamomum camphora* Nees., which is cultivated widely in Japan and Formosa and has also been found to thrive in India.

Preparation

(1) *Natural Camphor.*—The chopped up branches of the camphor tree are distilled in steam. The crude camphor thus obtained is refined by mixing with charcoal or lime and subsequent sublimation. The flowers of camphor thus obtained are then pressed into cakes.

(2) *Artificial Camphor.*—The oil of turpentine, which consists mainly of pinene, is dried, well-cooled and treated with a current of dry HCl when pinene hydrochloride is precipitated as a crystalline mass. The pinene hydrochloride is converted first into camphene by treating with an alkali and the camphene is then converted into isobornyl acetate by heating with glacial acetic acid. The isobornyl acetate gives *borneol* on hydrolysis. This borneol is then oxidized by chromic acid to camphor.



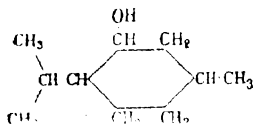


Properties and Uses.—Camphor is obtained in colourless crystals. It is lighter than water. It melts at 175° and boils without decomposition at 209° . It volatilizes when exposed to the air and when heated sublimes without a residue. It is inflammable and burns with a smoky flame. It is very slightly soluble in water (about 1 : 1000) but dissolves easily in absolute alcohol, ether, chloroform, glacial acetic acid, etc. The natural product is dextrorotatory while the synthetic one is inactive or r-form. Natural borneol which is a secondary alcohol is also dextrorotatory.

Camphor is used in medicine as a cardiac and respiratory stimulant. *Camphor water* is simply a 0.1 per cent solution in water used largely as a carminative and flavouring agent. *Spirits of camphor*, a 10 cent solution of camphor (w/v) in 90 per cent alcohol, is used in summer diarrhoea. Camphor is also used externally as a liniment. The use of camphor in the manufacture of celluloid and in some varieties of smokeless powder is of much industrial importance.

Menthol, 5-Methyl-2-isopropyl hexahydrophenol, $\text{C}_{10}\text{H}_{20}\text{O}$

This is the chief constituent of *peppermint oil*, the essential oil obtained from the plant *Mentha piperita* L. found in Europe and America as also from *Mentha arvensis* var. *piperascens* Holmes found in Japan. It separates in crystals on cooling the oil. It can be purified by distillation and subsequent crystallization from an organic solvent like ether, benzene, etc.



Pure menthol crystallizes in colourless needles, m.p. 44° , b.p. 215° . It has a sp. gr. of 0.881 at 45° . It is very

soluble in water, but dissolves easily in alcohol, ether, etc. The natural product has a sp. rotation $[\alpha]_D = -50^\circ$ (10 per cent alcohol). Being a secondary alcohol, it is not soluble in aqueous alkales and is thus distinguished from phenols such as thymol or catbolic acid. It is commonly employed as a carminative and an external remedy for neuralgic headache.

Resins.—Closely associated with the essential oils and terpenes are the resins. The resins are the natural secretions of plant tissues, the exudation being either normal or under pathological conditions.

They are all amorphous and vary in consistency from brittle solids to viscous masses. They are insoluble in water and are soluble in alcohol and to a great extent in ether but they are almost insoluble in petroleum ether. They are soluble in hot fixed oils. Even when freed from adhering or dissolved impurities, the resins consist of mixtures of various substances such as acids, alcohols, esters or neutral substances most of them of very complex composition.

Resins mixed with essential oils are known as *oleo-resins*, *ba'sams* or *turpentine*s ; e.g., canada balsam, commonly used in microscopy, copaiba, etc. Resins which are mixed with gums are known as *gum-resins* ; e.g., asaphœtida.

Many of the resins are used in medicine, such as *gum-benzoin*, *balsam of to'u*, *copaiba*, etc. The well-known purgatives such as *podophyllum*, *jalap*, etc., also belong to this class. Resins are largely used in the preparation of varnishes, in the making of some kinds of soap, and in sizing papers. Solution of rosin in hot castor oil is very sticky and is used in making 'fly-papers.'

PART IV

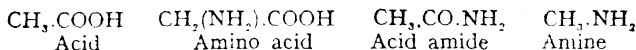
MISCELLANEOUS

CHAPTER XXIX

THE AMINO ACIDS

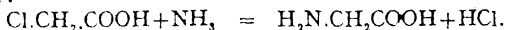
The study of the amino acids must prove to be of the greatest interest to the medical student as these are the units largely concerned in the make up of protoplasm and so intimately associated with the phenomenon of life. Whenever we break up a protein either by the action of suitable ferments or by the action of alkalis or dilute mineral acids we obtain these comparatively simple chemical substances.

Chemically, an amino acid may be considered to be derived from an organic acid in which one or more H atoms in the chain are replaced by an amino (NH_2) group, although there may be one or two exceptions, *e.g.*, proline or hydroxyproline. The nomenclature of these amino acids depends on the position of the NH_2 group in the chain. Thus, we have α -amino propionic acid, $\text{CH}_3\text{CH}(\text{NH}_2)\text{COOH}$, β -aminobutyric acid, $\text{CH}_3\text{CH}(\text{NH}_2)\text{CH}_2\text{COOH}$, etc. The amino acids should not, however, be confused with the acid amides or amines.

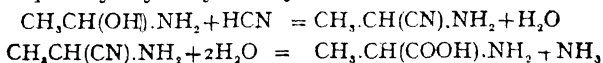


General Methods of Synthesis

(1) By heating the monohalogen fatty acids with ammonia:



(2) By the action of hydrocyanic acid on aldehyde ammonias forming nitriles of the amino acids which are subsequently hydrolyzed by HCl :



This method is simplified by treating the aldehyde with an aqueous or alcoholic solution of NaCN and NH_4Cl . The NaCN solution gives HCN and NaOH by hydrolysis and the NaOH evolves NH_3 from NH_4Cl .

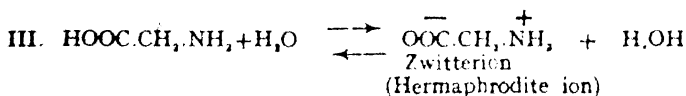
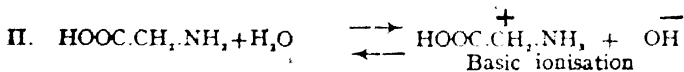
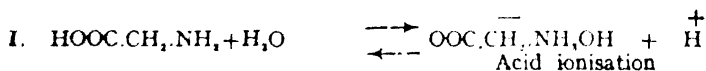
(3) By the reduction of ketonic acids in presence of NH_3 : e.g.,



General Properties and Reactions of Amino Acids

The amino acids are colourless crystalline bodies having a high melting point which is often indefinite. Many of them possess a sweet taste. They are usually fairly soluble in water but insoluble in ether or alcohol. All the naturally occurring amino acids with the exception of glycine are optically active.

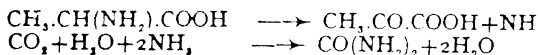
They are *amphoteric* (Gk. *amphoterōs*—on both sides) in reaction as they contain both carboxyl and amino groups. Thus they act either as weak acids or as weak bases. With an acid (HCl), glycine, for example, would form the salt $\text{CH}_2(\text{NH}_2\cdot\text{HCl})\text{COOH}$ and with an alkali (NaOH) it would also form the salt $\text{CH}_2(\text{NH}_2)\text{COONa}$. Glycine may thus be expected to ionise as follows:—



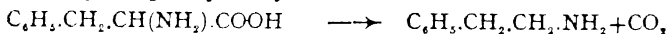
On the addition of an alkali, an *amphoteric electrolyte* (*ampholyte*) liberates H ions and behaves as an acid (I), and on the addition of an acid it liberates OH ions and behaves as a base (II). The splitting off of H or OH ions by an ampholyte depends, therefore, on the concentration of H ions of the solution. There is thus for each ampholyte a certain concentration of H ions at which its molecule will carry the same number of units of positive and negative

charges. The molecule as a whole will then be electrically neutral and will not migrate in an electric field either towards the cathode or towards the anode. The particular PH at which this phenomenon occurs is called the **isoelectric point** of the ampholyte. At the isoelectric point the ampholyte exhibits a minimum solubility.

The fate of amino acids in the animal organism must also arouse interest associated as they are with the proteins, thus forming an essential part of our food. It is not possible to discuss this question here but it may be stated that usually the amino acids are deaminized into the corresponding hydroxy acids or keto acids and the ammonia given off forms urea with carbonic acid: *e.g.*,

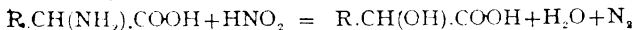


Under bacterial action, amino acids split off CO_2 and are converted into amines, *e.g.*, histidine gives histamine, phenylalanine gives phenyl ethyl amine, and so on:



Esters of amino acids are prepared by heating them with alcohol and passing dry HCl gas through the solution. The importance of the esters lies in the fact that they can be distilled under reduced pressure, and fractional distillation of these esters has been used for the separation of the individual amino acids from mixtures.

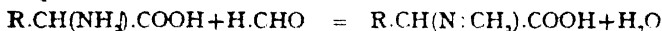
Nitrous acid reacts on amino acids forming hydroxy acids with liberation of nitrogen, the actual amount of nitrogen evolved being double that contained in the amino acid: *e.g.*,



This reaction has been utilized for the estimation of the amino nitrogen in the products of protein hydrolysis (*Van Slyke's Method*).

The NH_2 group of an amino acid condenses with *formaldehyde* forming a methylene derivative. This destroys the basic property of the NH_2 group but does not interfere with the acid reaction of the COOH group. This reaction has been employed for the quantitative estimation of the amount of amino acid nitrogen present, titration of the

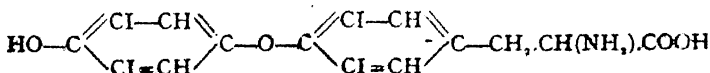
COOH groups being carried out by a standard alkali using phenolphthalein as an indicator (*Sorensen's Method*).



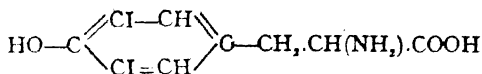
Classification of the Amino Acids

CLASS A. MONOAMINO MONOCARBOXYLIC ACIDS

1. *Glycine* or Amino acetic acid.
 $CH_3(NH_2).COOH$
2. *Alanine* or α -Amino propionic acid,
 $CH_3.CH(NH_2).COOH$
3. *Valine* or α -Amino isovaleric acid.
 $(CH_3)_2.CH.CH(NH_2).COOH$
4. *Leucine* or α -Amino isocaproic acid,
 $(CH_3)_2.CH.CH_2.CH(NH_2).COOH$
5. *Norleucine* or α -Aminocaproic acid,
 $CH_3.(CH_2)_3.CH(NH_2).COOH$
6. *Isoleucine* or β -Methyl- β -ethyl- α -amino propionic acid,
 $C_2H_5.CH(CH_3).CH(NH_2).COOH$
7. *Phenyl alanine* or β -Phenyl- α -amino propionic acid,
 $C_6H_5.CH_2.CH(NH_2).COOH$
8. *Tyrosine* or β -Parahydroxyphenyl- α -amino propionic acid,
 $HO.C_6H_4.CH_2.CH(NH_2).COOH$
9. *Threonine* or α -Amino- β -hydroxy butyric acid,
 $CH_3.CH(OH).CH(NH_2).COOH$
10. *Serine* or β -Hydroxy- α -amino propionic acid,
 $HO.CH_2.CH(NH_2).COOH$
11. *Thyroxine* or Tetra-iodo derivative of p-hydroxyphenyl ether of tyrosine,



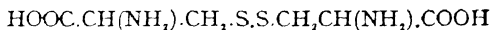
12. *Iodogorgoic acid* or Di-iodo derivative of tyrosine,



13. *Methionine* or γ -Methyl thiol- α -amino butyric acid,
 $\text{CH}_3.\text{S}.\text{CH}_2.\text{CH}_2.\text{CH}(\text{NH}_2).\text{COOH}$

14. *Cysteine* or β -Thiol- α -amino propionic acid,
 $\text{HS}.\text{CH}_2.\text{CH}(\text{NH}_2).\text{COOH}$

15. *Cystine, Dicysteine* or Di- (β -thiol- α -amino propionic acid),



CLASS B. MONOAMINO DICARBOXYLIC ACIDS

1. *Aspartic acid* or Amino succinic acid,
 $\text{HOOC}.\text{CH}_2.\text{CH}(\text{NH}_2).\text{COOH}$

2. *Glutamic acid* or α -Amino glutaric acid,
 $\text{HOOC}.\text{CH}_2.\text{CH}_2.\text{CH}(\text{NH}_2).\text{COOH}$

3. β -Hydroxy glutamic acid,
 $\text{HOOC}.\text{CH}_2.\text{CH}(\text{OH}).\text{CH}(\text{NH}_2).\text{COOH}$

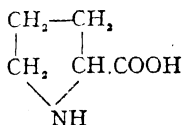
CLASS C. DIAMINO MONOCARBOXYLIC ACIDS

1. *Lysine* or α -, Σ -Diamino caproic acid,
 $\text{H}_2\text{N}.\text{CH}_2.(\text{CH}_2)_3.\text{CH}(\text{NH}_2).\text{COOH}$

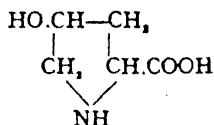
2. *Arginine* or α -Amino- δ -guanido valeric acid,
 $\text{H}_2\text{N}.\text{C}(:\text{NH}).\text{NH}.\text{CH}_2.\text{CH}_2.\text{CH}_2.\text{CH}(\text{NH}_2).\text{COOH}$

CLASS F. HETEROCYCLIC AMINO ACIDS

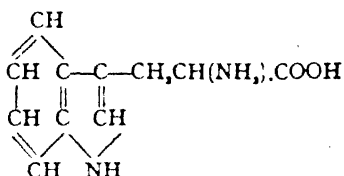
1. *Proline* or α -Pyrrolidine carboxylic acid,



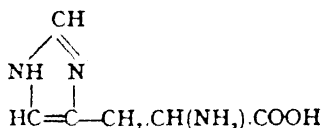
2. *Hydroxy proline* or β' -Hydroxy- α -pyrrolidine carboxylic acid,



3. *Tryptophane* or β -Indole- α -amino-propionic acid,



4. *Histidine* or β -Iminazole- α -amino propionic acid,



It may be remarked here that in Class A the amino acids, which contain only one NH_2 group and one COOH group, are neutral in reaction, in Class B the amino acids, which contain one NH_2 and two COOH groups, are acidic and in Class C the amino acids, which contain two NH_2 groups and only one COOH group, are basic in reaction.

The following ten amino acids, arranged alphabetically, are stated to be nutritionally *essential amino acids* indispensable for the growth and health of a growing animal or human being. They are not synthesized in the body and must therefore be supplied in the diet:

- | | |
|---------------|-------------------|
| 1. Arginine | 6. Methionine |
| 2. Histidine | 7. Phenyl alanine |
| 3. Isoleucine | 8. Threonine |
| 4. Leucine | 9. Tryptophane |
| 5. Lysine | 10. Valine |

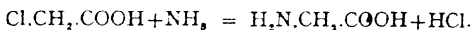
INDIVIDUAL AMINO ACIDS

Glycine, Glycocoll, Amino acetic acid, $\text{CH}_2(\text{NH}_2).\text{COOH}$

Occurrence.—It occurs in nature as a constituent of many proteins, gelatin and silk fibroin being specially rich in this compound.

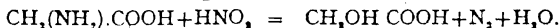
Synthesis and Preparation

It may be obtained synthetically by the action of ammonia on monochloroacetic acid:



It is usually prepared by hydrolyzing gelatin with hot concentrated HCl. The hydrolytic product is concentrated in vacuo, dissolved in absolute alcohol and converted into ethyl esters by passing dry HCl gas. On cooling, the glycine ester hydrochloride crystallizes out. The ester obtained from it is dried and distilled in vacuo. The amino acid obtained from the pure ester is recrystallized from water.

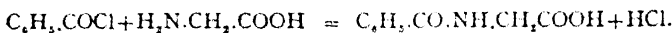
Properties and Reactions.—Glycine crystallizes from water in flattened colourless prisms. It becomes brown at about 228° and melts at 232° – 236° . It is insoluble in absolute alcohol and ether and is optically inactive. It is easily soluble in water and possesses a sweetish taste, whence the name glycocoll (*glycus*—sweet, *kolla*—glue). It is amphoteric in reaction. As an acid it forms salts with bases, the copper salt, $\text{Cu}(\text{CH}_2.\text{NH}_2.\text{COO})_2.\text{H}_2\text{O}$, being especially characteristic, crystallizing in dark blue needles. As a base it forms salts with acids, $\text{CH}_2(\text{NH}_2.\text{HCl}).\text{COOH}$ and these salts are generally crystalline and soluble in water. Glycine when acted on by nitrous acid yields glycollic or hydroxy acetic acid and nitrogen is evolved:



With absolute alcohol in the presence of dry HCl it forms the ethyl ester $\text{H}_2\text{N}.\text{CH}_2.\text{COOC}_2\text{H}_5$, which is an oily liquid with a peculiar smell and is soluble in ether and alcohol.

Hippuric Acid, Benzoyl glycine, $\text{C}_6\text{H}_5.\text{CO}.\text{NH}.\text{CH}_2.\text{COOH}$. This derivative of glycine occurs normally in

the urine of herbivora and is also excreted in the human urine if benzoic acid is taken by the mouth. The excretion of hippuric acid after the oral administration of sodium benzoate is used as a liver function test. Hippuric acid can be prepared by treating glycine in aqueous solution with sodium bicarbonate and benzoyl chloride:



It is a colourless crystalline substance, m.p. 187.5° . It is scarcely soluble in cold water but readily so in hot water, in alcohol and in ethyl acetate.

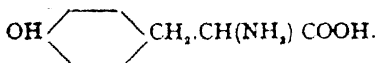
Leucine, α -Amino isocaproic acid,



This amino acid can be prepared by hydrolyzing casein with dilute sulphuric acid. It is one of the essential amino acids and is found in fair amounts in lactalbumin, in the globin of hæmoglobin, etc.

Leucine crystallizes in plates which are white and glistening, m.p. 295° - 297° . It is slightly soluble in cold water and to some extent in hot dilute alcohol. In watery solution it is lævorotatory; in HCl solution it is dextro-rotatory.

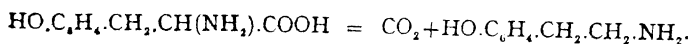
Tyrosine, *p*-Hydroxyphenyl alanine,



Occurrence and Preparation.—This occurs in most animal proteins and may form over 10 per cent of the products of hydrolysis. The best source is silk and caseinogen. In the free state it may be present in very small amounts in all tissues. It can be prepared by hydrolyzing caseinogen with 30 per cent sulphuric acid. The sulphuric acid is removed by barium hydroxide and the filtrate is concentrated, when the tyrosine separates out. It is purified by recrystallization from hot water.

Properties and Reactions.—It crystallizes from water in bunches of colourless needles, which melt at 314° - 318° . It is only slightly soluble in cold water but more so in hot

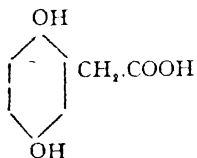
water. It is insoluble in absolute alcohol, acetone or ether. It is laevorotatory in aqueous solution, either acid or alkaline. When heated with Million's reagent it gives a red colour. When heated with nitric acid it gives a yellow colour which turns to orange on making alkaline with ammonia. On heating tyrosine in vacuo or as a result of putrefactive decomposition *tyramine*, a strongly physiologically active amine occurring in ergot, is formed and CO_2 is evolved:



Both leucine (as rounded discs) and tyrosine (as needle shaped crystals) are found in the urine in acute yellow atrophy of the liver (*Icterus gravis*).

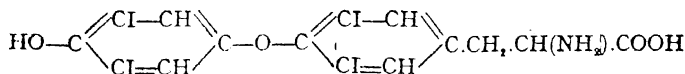
Derivatives of Tyrosine

(1) **Homogentisic Acid**, *Dihydroxyphenyl acetic acid*: It appears in the urine in the condition known as *alkaptonuria*; the urine darkens on the surface on standing and this is the characteristic feature of alkaptonuric urine. As homogentisic acid reduces Fehling's solution, it may be mistaken for glucose.

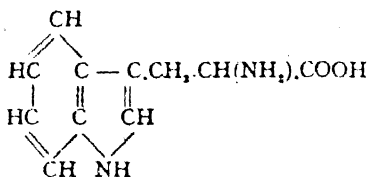


(2) Thyroxine, $\text{C}_{15}\text{H}_{11}\text{O}_4\text{NI}_4$

This is the active principle of the thyroid gland isolated by Kendall in 1914. Harrington and Barger (1923) have proved by synthesis that thyroxine is a tetra-iodo derivative of p-hydroxy phenyl ether of tyrosine having the structure,



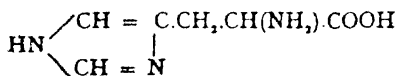
The content of thyroxine in fresh thyroid glands is about 0.03 per cent and thyroxine is stated to increase the oxidative processes in the body especially for the oxidation of fats and proteins.

(3) **Adrenaline** (see p. 248).**Tryptophane**, β -Indole- α -amino propionic acid.

It is a constituent of most proteins. It is prepared most conveniently from casein. It crystallizes from dilute alcohol in colourless plates, which are moderately soluble in cold water but more easily in hot water.

It is insoluble in absolute alcohol and ether. On heating it turns brown at 240° and melts at 252° ; when heated further it decomposes and forms indole and skatole. It is laevorotatory in aqueous solution but dextrorotatory in acid or alkaline solutions.

Tests. (1) If bromine water is added drop by drop to an aqueous solution of tryptophane made slightly acid with acetic acid a reddish violet colour is obtained; when shaken with amyl alcohol, it dissolves out the colouring matter; (2) If some concentrated sulphuric acid containing a little ferric sulphate is allowed to run down the side of an aqueous solution of tryptophane containing a little formaldehyde, a bluish violet colour is formed at the junction.

Histidine, β -Iminazole- α -amino propionic acid, $\text{C}_6\text{H}_7\text{O}_2\text{N}_3$ 

This amino acid contains the heterocyclic iminazole nucleus. It is a constituent of many proteins, and can be conveniently prepared from defibrinated blood or red blood corpuscles. It crystallizes from aqueous alcohol in colourless platelets, melting with decomposition at 253° . $[\alpha]_D = -39.74^\circ$. When heated in vacuo or when subjected to putrefactive decomposition it forms the active amine *histamine* (see p. 248). When heated with bromine water, a wine red colour is gradually developed (*Knoop's Reaction*).

CHAPTER XXX

THE PROTEINS

Composition of Proteins

The proteins are the essential constituents of all living cells and from the chemical standpoint they may be said to be composed of amino acid units or their derivatives condensed together. The word is derived from Gk. *protos*—first, as it was believed to be the first and most important component of living things. They all contain carbon, hydrogen, oxygen and nitrogen. Many of them contain sulphur and some contain phosphorus in addition. Iron is an essential constituent of hæmoglobin, the protein pigment of blood.

The proteins thus differ from the fats and carbohydrates in their containing nitrogen as an essential element and in fact proteins are differentiated from the other foods by grouping them as *nitrogenous foods*.

The *percentage composition* of a protein varies a great deal and the following figures may be taken as a rough average:

Carbon, 51 to 55; hydrogen, 6 to 7; oxygen, 21 to 23; nitrogen, 15 to 17; sulphur, 0.3 to 2.5; phosphorus, 0.1 to 1.0.

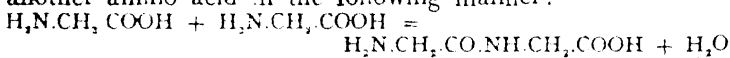
General Characters of the Proteins

The proteins are usually tasteless, odourless and colourless substances. When dry they are amorphous but some, such as egg albumin, have been prepared in the crystalline state. Many of them are soluble in water or in dilute salt solutions or in very dilute acids or alkalies. Their aqueous solutions show colloidal properties owing to their possessing very high molecular weights. The molecular weight of hæmoglobin, $C_{758}H_{1203}O_{218}N_{195}FeS_3$, has been estimated to be about 16,000 and many proteins show molecular weights varying from 16,000 to 60,000 while the protein globulin

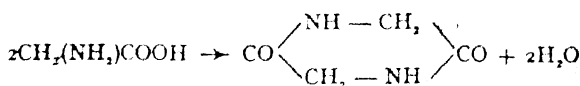
obtained from thyroid gland has been proved to possess a molecular weight as high as 650,000, and even higher figures have been obtained in some other proteins. The proteins are all optically active. Owing to the presence of some free amino and free carboxyl groups they are *amphoteric* in nature, combining either with bases or acids according to the pH of the solution. On hydrolysis with dilute acids or alkalis or by some proteolytic enzymes they are ultimately broken down into the component amino acids.

Structure of Proteins

It has been mentioned above that the proteins are composed of amino acid units condensed together. The amino group of an amino acid condenses with the carboxyl group of another amino acid in the following manner:



This linking of a COOH group with an NH₂ group forming the group—CO.NH—(similar to the formation of an acid amide) is known as a '*peptide linkage*.' When two amino acids, which may be similar or dissimilar, combine together we get a *dipeptide*, when three amino acids combine together we get a *tri-peptide*, and so on. A large number of di- tri-, and higher *poly-peptides* have been synthesized in the laboratory and their properties have been found to be similar to the polypeptides obtained from natural proteins. It should not, however, be assumed that this *amide type of linking* is the only one possible, and complicated cyclic types of linking such as a substituted diketopiperazine, are also believed to exist:



General Reactions of Proteins

(1) Colour Reactions

(a) *Millon's Reagent*.—On adding Millon's reagent to a solution of a protein, a white precipitate is formed, which on heating turns pink to brick-red. The colour reaction is due to the presence of the hydroxyphenyl group (found in

the amino acid tyrosine) in the protein molecule. The reagent consists of 30 c.c. of mercury dissolved in 570 c.c. concentrated nitric acid and then diluted with two volumes of water.

(b) *Xanthoproteic Reaction*.—On heating a solution of a protein with concentrated nitric acid a yellow colour is produced, which changes to orange on the addition of strong ammonia or caustic soda solution. The reaction is due to the presence of the phenyl group in the protein molecule which forms a nitro compound. The amino acids possessing this group are tyrosine, phenyl alanine and tryptophane which are the components of common proteins.

(c) *Glyoxylic Acid reaction* (or *Hopkins—Cole reaction*).—On the addition of a solution of glyoxylic acid to the protein solution followed by the addition of a bottom layer of conc. sulphuric acid by means of a pipette, a reddish violet ring is formed at the junction of the two liquids. This reaction is due to the presence of tryptophane and is not given by gelatin which does not contain this amino acid. *Glyoxylic acid reagent* is prepared by reducing a saturated solution of oxalic acid with powdered metallic magnesium. The solution is kept cool, filtered from the magnesium oxalate, acidified with acetic acid and then diluted with water. Glyoxylic acid is also known as glyoxalic acid.

(d) *Biuret Test*.—If a little of a 5 per cent solution of NaOH is added to a protein solution and then one or two drops of a dilute copper sulphate solution (1 per cent), a violet colour appears. If the solution of the protein is hydrolyzed, the colour may be pink due to the presence of proteoses or peptones. This reaction is given by all substances containing two —CO.NH— groups, either attached to one another or to the same carbon atom or to the same nitrogen atom (*cf. urea*).

(2) Coagulation Reactions

(a) *Heat*.—If a solution of a protein is faintly acidulated with a few drops of acetic acid and heated, a flocculent irreversible precipitate of the protein is obtained. This is an important test for albumin in the urine. The presence

of strong acids or alkalies retards coagulation and the presence of excess of neutral salts hastens it.

(b) *Nitric Acid*.—If strong nitric acid is added carefully by means of a pipette to a protein solution so as to form a layer, a white ring of coagulum will form at the junction of the two solutions. This is also a common test for detecting proteins in the urine and is known as *Heller's Test*.

(3) **Precipitation Reactions**

(a) *Concentrated Neutral Salts*.—All proteins, except peptones, are precipitated from their solution by saturation with ammonium sulphate crystals. Magnesium sulphate, sodium sulphate, and sodium chloride are also employed for this purpose (see albumins and globulins). This is a reversible precipitate, which after having been filtered off may be dissolved again in its original form. For example, a solution of globulin in NaCl if dialyzed is precipitated and redissolves if NaCl is again added to it.

(b) *Salts of Heavy Metals*.—Copper sulphate, lead acetate, mercuric chloride, etc., form precipitates with proteins.

(c) *Alkaloidal Reagents*.—Reagents, such as phosphotungstic acid, trichloroacetic acid, picric acid, sulphosalicylic acid, potassium mercuric iodide (Mayer's reagent), tannic acid, etc., form precipitates with proteins in an acid solution.

Estimation of Proteins in Urine.—It is often essential for clinical purposes to form some estimate of the amount of protein, say albumin, in the urine. This is easily done in the simple apparatus known as *Aufrecht's albuminometer* which is a modification of the old fashioned Esbach's albuminometer. It is a centrifuge tube graduated to show the percentage of albumin and there are marks to show the level of urine and reagent to be taken. The reagent is the same as *Esbach's reagent* which is a 1.5 per cent solution of picric acid with 3.0 per cent citric acid. After adding the reagent the tube is centrifuged at 3000 revolutions per minute for 2½ minutes and the percentage of albumin found is read directly. The Esbach's method of estimation requires 24 hours.

Classification of Proteins

- | | |
|--------------------------|-----------------------------|
| 1. Protamines | 9. Conjugated Proteins |
| 2. Histones | (a) Nucleoproteins |
| 3. Albumins | (b) Mucoproteins |
| 4. Globulins | (c) Chromoproteins |
| 5. Glutelins | 10. Derivatives of Proteins |
| 6. Gliadins or Prolamins | (a) Metaproteins |
| 7. Scleroproteins | (b) Proteoses |
| 8. Phosphoproteins | (c) Peptones |
| | (d) Polypeptides |

I. Protamines, *e.g.*, salmine, clupeine, etc. These are the simplest proteins known to occur in nature. They contain a high percentage of nitrogen (25-30) but no sulphur or phosphorus. They are all found in the sperm of fishes such as salmon, herring, etc. They are easily soluble in water and their solutions are strongly alkaline. They are also soluble in dilute ammonia and give a pink biuret reaction (*cf.* peptones); they are not coagulated on heating. On hydrolysis, they yield a very large proportion of diamino acids.

II. Histones, *e.g.*, thymus histone, globin (from hæmoglobin), &c. They are soluble in water and the solutions are alkaline. The basicity is intermediate between protamines and albumins and they yield a larger proportion of diamino acids than albumins. They are not soluble in dilute ammonia and are precipitated from aqueous solutions by caustic alkalis or by ammonia. They are not coagulated by heat except in the presence of a salt.

III. Albumins, *e.g.*, egg-albumin, serum-albumin, lactalbumin, etc. They are soluble in pure water and in dilute salt solutions and are coagulable by heat. Their solutions are almost neutral in reaction. They are precipitated from their solutions by complete saturation with ammonium sulphate or zinc sulphate but are not precipitated by saturating their neutral solutions with sodium chloride or magnesium sulphate. They respond to *Thymol-Test* for carbohydrates (see p. 195). They contain a high percentage of sulphur. They are lævorotatory, *e.g.*, egg albumin $[\alpha]_D = -35^\circ$, serum albumin $[\alpha]_D = -56^\circ$.

IV. **Globulins**, *e.g.*, ovo-globulin (from egg yolk), serum-globulin, etc. These are insoluble in pure water, but soluble in dilute solutions of neutral salts (such as NaCl) and in alkaline carbonates and are coagulated by heat. They are precipitated from their solutions by half-saturation with ammonium sulphate and also by complete saturation with sodium chloride or magnesium sulphate and from weak alkaline solution by CO_2 .

V. **Glutelins**, *e.g.*, glutenin (from wheat). These proteins have been found only in plants. They are insoluble in neutral water but are readily soluble in very dilute acids or alkalis.

VI. **Gliadins** (Prolamins), *e.g.*, gliadin (from wheat), zein (from maize), hordein (from barley). They are called prolamins as they contain a relatively large proportion of the amino acid proline. They are insoluble in water, absolute alcohol and other neutral solvents but are soluble in 70 per cent alcohol. They occur only in plants.

VII. **Scleroproteins**, *e.g.*, keratin (from horn, feather, hair, nails, etc.), elastin (ligaments), collagen (cartilage and bone), etc. They are found only in animal tissues and are often referred to as *albuminoids*. They are insoluble in all neutral solvents and resist the action of proteolytic enzymes. Keratins are soluble in alkalis but insoluble in dilute acids.

N.B. *Gelatin* is obtained by boiling bones with water when collagen, the protein present, undergoes hydrolytic changes and yields the soluble product gelatin, which sets to a jelly on cooling and liquefies on warming.

VIII. **Phosphoproteins**. *e.g.*, casein (from milk) and vitellin (from egg-yolk). These are compounds of a protein molecule with a phosphorus-containing compound other than nucleic acid or lecithin. It is probable that the phosphorus is in combination with one of the amino acids. They are distinctly acid in reaction and are insoluble in water, dilute acids and in half saturated solution of ammonium sulphate but are soluble in dilute alkali; this solution does not coagulate on heating.

IX. Conjugated Proteins.

1. **Nucleoproteins.**—In this class the protein molecule is combined with a *nucleic acid*. A nucleic acid consists of a carbohydrate, phosphoric acid, two *purine* bases and two *pyrimidine* bases. Nucleoproteins are the main constituents of cell nucleus and have been prepared from yeast and from animal tissues like thymus, pancreas, etc. They are insoluble in water but dissolve in dilute alkalis and in concentrated acids. They are acidic in character and are coagulated by heat. As uric acid is synthesized from nucleic acid in the body, foods rich in nucleoproteins such as liver, kidney, pancreas, fishroe, etc., are contraindicated in gouty subjects.

2. **Mucoproteins.**—The protein molecule is combined with mucoitin sulphuric acid or chondroitin sulphuric acid. The latter consists of sulphuric acid, acetic acid, glycuronic acid, and glucosamine or galactosamine. *Mucins*, pseudo-mucins and mucoids belong to the class of mucoproteins. The mucins are soluble in water and in alkaline carbonates and in ammonia, and are precipitated from these solutions by acetic acid. Mucin is present in saliva and in all secretions from mucous surfaces of the body. It is almost always present in the urine, and as it is readily hydrolyzed to glucosamine (see page 210), glycuronic acid, etc., urine containing excess of mucin slightly reduces Fehling's solution and may thus be mistaken for a reducing sugar. In such cases, a previous separation of mucin by acetic acid and filtration removes the fallacy. Mucoids are found in bone, tendon, etc.

3. **Chromoproteins.**—These are combinations of proteins with a colouring matter. The best example is hæmoglobin found in the blood of all vertebrates and of some invertebrates. Hæmoglobin consists of the histone globin, and the iron-containing colouring matter *hæmatin*.

X. Derivatives of Proteins

When the complex molecule of a protein is submitted to the action of hydrolytic agents such as enzymes, mineral acids, or caustic alkalis, a gradual disintegration of the

molecule takes place. The different *stages of hydrolysis* are as follows: metaproteins, proteoses, peptones, polypeptides, and amino acids.

1. Metaproteins.—This partially hydrolyzed product still retains most of the characteristics of the protein. According as an acid or alkali is used as the hydrolyzing agent, the product is referred to as an *acid*—or *alkali*—*metaprotein*. For instance, an acid metaprotein can be prepared from egg-white (30 c.c.) by treating this with glacial acetic acid (10 c.c.) and allowing to stand for 24 hours. The jelly formed is diluted with water and the solution of the metaprotein is filtered off from the unattacked protein.

Metaproteins are coagulated by heat in neutral solution; they are insoluble in water but soluble in dilute acids or alkalis, and precipitated by neutralization; they are also precipitated by half-saturation with ammonium sulphate or by complete saturation with magnesium sulphate.

2. Proteoses.—At this stage of hydrolysis, the product is no longer coagulated by heat but it is still capable of being salted out by complete saturation of its solution by ammonium sulphate. They are readily soluble in water and diffuse through a dialyzer very slightly. They give a pink biuret reaction. Proteoses are precipitated by a saturated solution of picric acid in the cold; the precipitate redissolves on heating but it reappears on cooling. This test is used to differentiate between *albumose* (a proteose) and *albumin* in urine.

The proteoses are divided into two classes: (1) *primary proteoses* which are completely precipitated by half-saturation with ammonium sulphate and (2) *secondary proteoses*, which are only salted out by complete saturation of their solutions with ammonium sulphate.

A mixture of proteoses and peptones can be prepared by treating egg-white (1 c.c.) with 0.4 per cent HCl (20 c.c.) and pepsin (0.01 g.) at 37° for several days. The proteoses can then be separated from the peptones by saturation with ammonium sulphate.

In certain diseases of the blood and bone marrow and also in other conditions, a proteose commonly known as

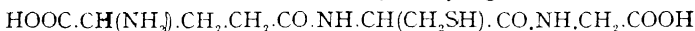
'Bence-Jones Protein' appears in the urine and is precipitated rendering the urine turbid or even milky on standing.

3. **Peptones.**—These are readily soluble in water and are not coagulated by heat nor are they salted out by complete saturation of their solution by ammonium sulphate. They also give a pink biuret reaction. They are precipitated by phosphotungstic acid, tannic acid and by lead acetate but not by other reagents which precipitate proteins. They diffuse through animal membranes readily.

The peptones are largely used in making culture media for bacteriological work; *Witte's peptone*, which is widely used for this purpose, is prepared by hydrolyzing fibrin with pepsin in presence of dilute HCl.

4. **Polypeptides.**—It has been mentioned before that the polypeptides are combinations of amino acids through the peptide linkage— CO.NH— . Substances containing 18 or 19 amino acids thus linked together have been synthesized in the laboratory, and the properties of these synthetic polypeptides resemble those obtained by the hydrolysis of proteins. They are solid and usually readily soluble in water and give the biuret reaction. The fact that nitrous acid does not yield much free nitrogen with polypeptides, shows that they do not contain many free amino groups of the amino acids.

Glutathione. *Glutamyl cysteinyl glycine,*



This is a tripeptide discovered in yeast and in animal tissues especially in the liver and other glands. It is closely associated with the processes of oxidation and reduction going on in the tissues. It can be prepared from yeast and forms a crystalline substance easily soluble in water.

Insulin

This is a crystalline protein found in the pancreas and is concerned in the metabolism of sugar. It reduces blood sugar when injected subcutaneously and is widely used in the treatment of diabetes. Crystalline insulin contains about 0.5 per cent of zinc in its molecule. The molecular weight is believed to be about 35,100. It is fairly stable in acid

but not in alkaline solution. The amino acids obtained by the hydrolysis of crystalline insulin (White) are: leucine (30%), glutamic acid (30%), cystine (12.5%), tyrosine (12%), histidine (8%), arginine (3%) and lysine (2%).

Proteins in Nutrition

The *function* of food proteins is (1) to replace the daily loss of body proteins, (2) to provide material for the building of tissue proteins during growth and convalescence, (3) to provide material for the formation of body enzymes and hormones such as pepsin, trypsin, insulin, adrenaline, thyroxine, etc., and (4) to provide energy through the transformation to carbohydrates and other oxidizable substances.

The minimum daily *requirement* of protein for nutrition is about 0.7 gm. per kgm. of body weight but 1 gram per kilogram of body weight is recommended, about half of which should be from animal source. A chronic deficiency of protein in the diet leads to a pathological condition known as "famine œdema".

Digestibility Coefficient of Proteins

Proteins taken as food are hydrolyzed to amino acids by proteolytic enzymes and are then absorbed in the blood stream, whereas the unhydrolyzed portion of the protein is excreted in the faeces. Certain proteins such as those of milk or eggs are easily digested and hydrolyzed into amino acids, whereas other proteins such as those of the pulses are only partially hydrolyzed during the same period.

The term digestibility coefficient of a protein is defined as the percentage of the ingested protein absorbed into the blood stream after the process of digestion is complete. Thus the digestibility coefficient of the proteins of rice, wheat and skimmed milk powder are 97, 93 and 90 per cent respectively whereas those of lentil and gram are 88 and 86 per cent respectively.

Biological Value of Proteins

It has been found that proteins from different sources differ a good deal as regards the nature and relative proportions of the different amino acids present, some containing more of the *nutritionally essential amino acids* (see page 364) than others. Out of the amino acids absorbed into the blood stream after the process of digestion, the body takes up only those that are required for its nutritional and physiological needs and the rest are discarded after deamination.

The term biological value of a protein is defined as the percentage of the absorbed protein utilized in meeting the nitrogen requirements of the body. The biological value of a protein is high if the nature of the amino acids present is similar to those of the body proteins and if it contains all the *essential amino acids*. The biological values of the proteins of some of our common foodstuffs

are: milk 92, whole eggs 94, rice 80, wheat 68, mutton 71, rohit fish 80, hilsa fish 72, mung dal 74, gram (chhola) 62, lentil (musur) 58, soya bean 64 and ground nut 57.

The biological value of a protein is determined from the following formula after estimating (a) the food nitrogen intake, (b) the total urinary nitrogen excreted, (c) the total faecal nitrogen excreted, (d) the endogenous urinary nitrogen, and (e) the endogenous faecal nitrogen:

$$\text{Biological Value} = 100 \times \frac{\text{body nitrogen saved}}{\text{food nitrogen absorbed}}$$

$$= 100 \times \frac{a - (c - e) - (b - d)}{a - (c - e)}$$

For example,

	Daily nitrogen intake gm.	Daily urinary nitrogen gm.	Daily faecal nitrogen gm.
Nitrogen-free diet	nil	1.499(d)	0.946(e)
Protein given	8.785(a)	3.435(b)	3.251(c)
Biological value = 100 ×	$\frac{8.785 - (3.251 - 0.946) - (3.435 - 1.499)}{8.785 - (3.251 - 0.946)}$		
	= 70 per cent.		

CHAPTER XXXI

THE VITAMINS

Classification, Occurrence, Preparation, Synthesis, Properties, Action, Assay

Introduction

Up till the beginning of the 20th Century, it was believed that the most essential constituents of our food were the *carbohydrates*, *fats* and *proteins*. From a careful study of the effect of different foodstuffs on animals, however, scientific workers gradually came to the conclusion that no animal could live upon a mixture of the above pure constituents, and that besides some *inorganic salts* and *water*, certain other constituents were essential for life, and these were at first called the "*accessory food factors*", of diet. These accessory food factors are now known as '*vitamins*'.

A vitamin may, therefore, be *defined* as a specific organic compound which is essential for the normal growth and maintenance of life of an animal, and which does not belong to any of the great class of foodstuffs such as carbohydrates, fats, proteins, and minerals.

The most significant fact about the vitamins is that only very small amounts of these are necessary compared to the large amounts of carbohydrates, fats and proteins required for our food. Thus for example, an ounce of vitamin D is probably enough to supply the daily requirements of one to two million people, whereas several ounces of carbohydrates, fats and proteins are necessary for each individual per day. And even with vitamin C, of which the daily requirement is higher than that of any other vitamin, the daily dose for an adult is probably as low as 50 milligrams. This fact indicates that the vitamins act like *catalysts* promoting chemical processes in cell life and therefore function quite differently from the other classes of foodstuffs, which are either necessary for the supply of energy or for the building up or repair of our body material.

Many of these vitamins which even in the near past were being prepared from *natural sources* at an enormous cost are now being increasingly produced *synthetically* at a

very low cost and has made mass feeding an economic possibility.

Classification

As the true chemical nature of the vitamins was unknown at the time of their discovery, they were first designated by alphabets, such as A, B, C, D, E, etc. They were also broadly divided into two main groups: (1) *fat-soluble vitamins*, such as vitamin A, vitamin D, vitamin E, etc., and (2) *water-soluble vitamins*, such as vitamins B, vitamin C, etc. These nomenclatures have still persisted although with the rapid advance of our scientific knowledge, the chemical constitutions of most of these have been elucidated and their proper scientific names substituted. The following *table* shows the classification and summarizes some of the broad facts about vitamins:

<i>Name of the vitamin</i>	<i>Corresponding deficiency disease in human beings</i>	<i>Scientific name of the compounds having vitamin activity</i>
<i>Fat-soluble</i> Vitamin A	Hemeralopia (night-blindness), xerophthalmia and keratomalacia, leading to blindness, phrynoderma (toad-skin)	Vitamin A, β -Ioninol
Provitamin A	Same as Vitamin A	α -, β -, and γ -Carotenes, and Cryptoxanthin
Vitamin D	Rickets in children and osteomalacia in adults	Vitamin D ₂ or calciferol, Vitamin D ₃ or activated 7-dehydrocholesterol, Vitamin D ₄ or activated 22-dihydroergosterol
Vitamin E	Sterility	α -, β -, and γ -Tocopherols
Vitamin K	Hæmorrhage (hypoprotrombinemia)	Vitamin K ₁ or phyllochinon, Vitamin K ₂ , Phthiocol
<i>Water-soluble</i> Vitamin B Complex, including: Vitamin B ₁	Beri-beri	Aneurin, Thiamin

<i>Name of the vitamin</i>	<i>Corresponding deficiency disease in human beings</i>	<i>Scientific name of the compounds having vitamin activity</i>
Riboflavin	Cheilosis, angular stomatitis, scrotal dermatitis, superficial keratitis	Riboflavin
Pellagra-preventive vitamin	Pellagra	Nicotinic acid, and Nicotinamide
Vitamin B ₆ (rat-pellagra factor)	Role in human nutrition not fully ascertained	Pyridoxin, Adermin
Pantothenic acid (chick-pellagra factor)	Role in human nutrition not known	Pantothenic acid
Vitamin H	Role in human nutrition not known definitely	Biotin
Vitamin C	Scurvy	Ascorbic acid, Cevitamic acid, Hexuronic acid.
Vitamin P	Nutritional purpura	

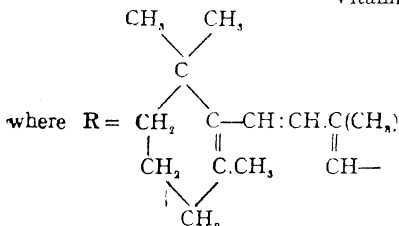
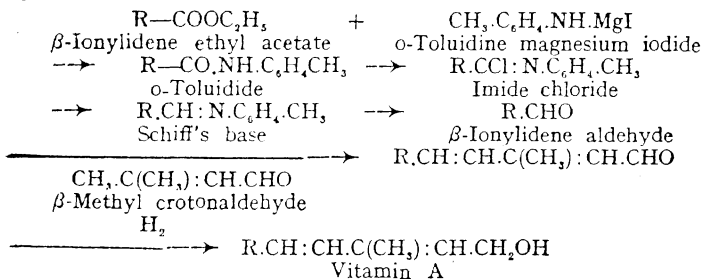
Vitamin A, β -Ioninol.

Occurrence.—It occurs only in the animal organism and in foods of animal origin. The International Unit (I.U.) of vitamin A is taken as the specific activity contained in 0.6 γ or μg (γ =*microgram*, i.e., one millionth of one gram; 1,000 μg =1 mgm.) of the standard preparation of pure β -carotene (roughly equivalent to 0.3 γ of pure vitamin A). Cod liver oil (850—2,500 I.U. per g.), halibut liver oil (19,000—36,000 per g.), and shark liver oil (4,000—15,000 per g.) are the richest sources of this vitamin. Many other fish liver oils including those of *mrigel*, *rohit*, *vetki*, *kalla*, etc., are also known to contain fair amounts. Mammalian liver is a good source, and the vitamin occurs in whole milk (160—225 I.U. per 100 grams), butter (3,500—5,000 per 100 g.), and egg (1,000—2,000 per 100 g.). Green leaves which contain the *precursors* of vitamin A (see Provitamins) have a high vitamin A value although they do not contain vitamin A itself.

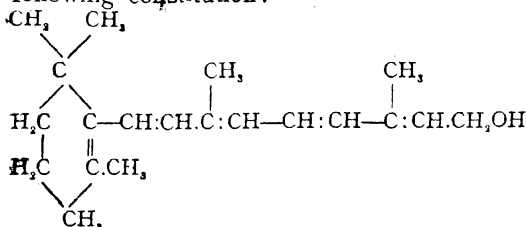
Preparation.—It is found in the unsaponifiable fraction of fish liver oils. Fish liver oil with a high vitamin potency is used as the starting material. The oil is distilled in very high vacuum (10^{-3} to 10^{-6} mm. Hg.) using a special

apparatus known as the 'cyclic molecular still', the process being known as 'molecular distillation'. A vitamin A concentrate of high potency (1-2 million I.U. per gram) is thus isolated. The concentrate is crystallised by fractional freezing and filtration in the cold, ethyl formate or propylene glycol being used as solvent. The vitamin found in the concentrate can also be purified by conversion into an ester, *e.g.*, vitamin A acetate, by treating with a solution of acetyl chloride in pyridine; the acetate is then extracted with petroleum ether.

Synthesis.—The following reactions have led to the synthesis of vitamin A:



Constitution, Physical & Chemical Properties.—Vitamin A or β -Ioninol, $\text{C}_{20}\text{H}_{30}\text{O}$, has been shown to possess the following constitution:



From methyl alcohol, it crystallizes in pale yellow plates, m.p. 8° , with the solvent of crystallization. From ethyl formate or propylene glycol it forms crystals, m.p. 63° - 64° . It distils in high vacuum (5×10^{-3} mm. Hg) at 120° - 125° . In the ultra-violet region the vitamin shows an absorption band at $328 \text{ m}\mu$ with an extinction coefficient of $E_{1\%,1\text{cm}} = 1725$. It is a primary alcohol and forms esters, and the acetate, m.p. 56° - 58° , being the most stable of these, is utilized for its purification. As a highly unsaturated compound, it is very susceptible to oxidation. On rapid aeration or exposure to sunlight or ultra-violet light it is rapidly destroyed, but it is fairly stable to high temperature in the absence of light or oxygen.

Physiological Action and Requirement.—Vitamin A is necessary for the regeneration of the visual purple of the retina of the eye and its deficiency causes a loss of efficiency of adaptation of vision to a changed intensity of light. Thus vitamin A deficient persons find it difficult to see objects in dim light. Its deficiency also causes (1) xerophthalmia and keratomalacia which ultimately lead to blindness, (2) phrynoderma, which is a type of dryness and hyperkeratosis of the skin, commonly known as 'toad skin', and (3) to a high incidence of respiratory diseases.

The vitamin A requirements for different ages are as follows: Adult man or woman 5,000 I.U., during pregnancy and lactation 6,000—8,000, children 1-12 years 1,500—4,500, boys and girls 12-18 years 5,000—8,000. If the source is carotene, the intake should be double the above values.

Methods of Assay

I. Biological

Young growing rats are given a diet containing all known essentials for growth except vitamin A until their reserves for this factor are exhausted and they cease to grow. This takes about 4 to 5 weeks. Different groups of these rats are then given the standard preparation of vitamin A and the sample to be tested at different levels of intake. After about 3 weeks, the average increase in the weights of the rats in the different groups are determined and a comparison of these figures allows the calculation of the activity of the sample in terms of the standard preparation.

II. Chemical or Physical

(1) *Colorimetric Method.*—The oil or fat is saponified in the cold with N/2 alcoholic potash and the mixture is extracted with

ether which takes up the vitamin A. The residue from the ether is taken for the assay. A 25 per cent solution of pure antimony trichloride in chloroform is added to a solution of the vitamin-containing sample in chloroform. A blue colour develops which begins to fade after 30 seconds. The intensity of colour is immediately measured with a Lovibond tintometer. The results are expressed in blue units, one Carr-Price Blue Unit being approximately equivalent to 55 I.U.

(2) *Spectrophotometric Method*.—The extinction coefficient of a solution of the unsaponifiable fraction of the material in pure cyclohexane is determined by a spectrophotometer. A one per cent solution of vitamin A gives, as stated before, an absorption band at 328 m μ with an extinction coefficient of 1725. If, for example, the extinction coefficient is found to be 1, the concentration of

vitamin A present in the sample will be $\frac{1}{1725}$ per cent or $\frac{1000 \times 1000}{1725}$ micrograms per 100 g. of the material.

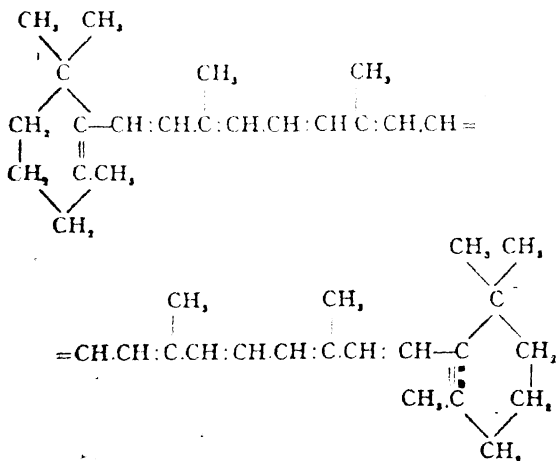
Provitamins A

Occurrence.—These precursors of vitamin A, which are converted into vitamin A in the body and can thus serve as the sources of vitamin A, occur in plant tissues and there are several compounds occurring in plants, all having the vitamin A activity. Of these, the most important are the α -, β -, and γ -carotenes and cryptoxanthine, β -carotene being the most potent (about twice as much as α - or γ -carotene). The following figures under brackets represent I.U. per 100 grams of substance: Green leafy vegetables, such as spinach (*palang-sak*) (5,600—6,500), mint (*pudina*) (3,300—4,600), *neem* (4,600), *sajina-sak* (11,300), amaranth or *notey-sak* (*Amarantus gangeticus*) (500—11,000), *dhane-sak* (coriander leaves) (10,460—12,600), *kalmi-sak* (*Ipomoea reptans*) (5,200—5,500), *kumra-sak* (*Cucurbita maxima*) (5,750—7,200), etc., are very good sources; carrots (2,200—4,000), green peas (1,000—1,300), etc., are fair sources. Fodder grasses such as 'doob' (lawn grass), Napier grass, wheat grass, etc., are good sources, and the leaves of the *water hyacinth* have been recently shown to be rich in this vitamin (3,000—3,500). Red palm oil is stated to be one of the richest sources (40,000—50,000). Small quantities of the substance are also known to occur in milk, butter, eggs, corpus luteum, etc.

Preparation.—The dry material is extracted in a Soxhlet with petroleum ether (b.p. 40°–60°C). The chlorophyll and xanthophyll are removed from the extract by adsorption with calcium diphosphate, and on evaporation of the solvent a mixture of the provitamins is left behind. The individual members of the mixture can be separated from each other by following the *chromatographic adsorption* technique, using various adsorbents such as aluminium oxide, magnesium oxide, sintered lime, etc., from which they are again eluted and purified further.

Synthesis.—Not known.

Constitution. Physical and Chemical Properties.—The provitamins are unsaturated hydrocarbons and are termed *carotenoids*. The structural formula of β -carotene, which is used as the standard, is as follows:



Although one molecule of β -carotene can theoretically give rise to two molecules of vitamin A by breaking down at the double bond marked with a dotted line, the animal organism is able to obtain only one molecule of vitamin A, due probably to an unsymmetrical fission of the molecule. This fact is of great practical importance, since we require twice as much of β -carotene as of vitamin A. The α - and β -carotenes and cryptoxanthine have only half the potency of

β -carotene. (1.2 γ of α - or γ -carotene or of cryptoxanthine = 0.6 γ of β -carotene = 0.3 γ of vitamin A).

The carotenes crystallize in red prisms, soluble in ordinary fat solvents but insoluble in water. They are easily destroyed by aeration or exposure to sunlight. Both β - and γ -carotenes are optically inactive whereas α -carotene is optically active. The melting points of α -, β -, and γ -carotenes and of cryptoxanthine are 187° , 184° , 178° and 169° respectively. β -carotene constitutes the major portion of the mixed carotenes prepared from various plant materials.

Physiological Action.—Same as with vitamin A.

Methods of Assay

Biological.—Same as with vitamin A.

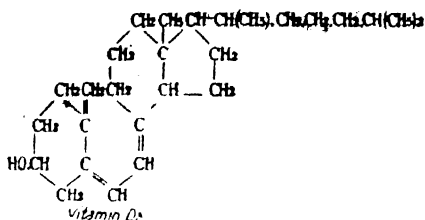
Chemical or Physical

(1) *Colorimetric Method.*—The material is finely ground with glass powder and then saponified at room temperature with alcoholic potash. The mixture is extracted with petroleum ether (b.p. 40° — 60°) and the ethereal extract is shaken up with 92 per cent methanol to remove xanthophylls. The provitamins remain in the petroleum ether and the colour is matched against a standard solution of β -carotene or against standard coloured solutions (e.g., azobenzene or potassium dichromate) with the help of a colorimeter. Or, the blue colour which develops with a solution of antimony trichloride in chloroform can be measured with a Lovibond tintometer.

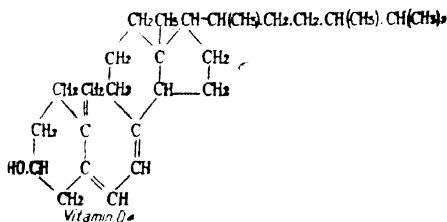
(2) *Spectrophotometric Method.*—The total amount of carotenes can also be determined spectrophotometrically and expressed in terms of β -carotene, using the wave length $450\text{ m}\mu$ of β -carotene, for which $E_{1\%}^{1\text{cm}} = 2500$ in petroleum ether

Vitamins D

Occurrence.—Of the different forms of vitamin D the following three are well-known: (1) vitamin D_2 or calciferol, (2) vitamin D_3 or activated 7-dehydrocholesterol, and (3) vitamin D_4 activated 22-dihydroergosterol. Fish liver oils are the richest natural sources of vitamin D_3 : blue fin tuna liver oil (40,000 I.U. per g.), cod liver oil (85—250 per g.), halibut liver oil (1,200 per g.), shark liver oil (100—150 per g.), etc. Vitamin D_3 occurs in nature in small quantities in a few foodstuffs like butter, eggs, mammalian liver, etc.: milk (0.01—0.02 per g.), butter (0.4—1.5 per g.), mammalian liver (0.15—



(Irradiated 7-dehydrocholesterol)



(Irradiated 22-dihydroergosterol)

The vitamins D are soluble in absolute alcohol, acetone, ether and other fat solvents. They are stable to heat and atmospheric oxygen when exposed for short periods. Calciferol (vitamin D₂) is a white crystalline substance, m.p. 115°-117° $[\alpha]_D^{20} = +82.6^\circ$ (acetone); vitamin D₃, m.p. 82-83°, $[\alpha]_D^{20} = +83.3^\circ$ (acetone); vitamin D₄, m.p. 107°-108°, $[\alpha]_D^{20} = 89.3^\circ$ (acetone). They all show characteristic absorption spectra. The 3,5-dinitrobenzoates show characteristic melting points and specific rotations.

Physiological Action and Requirement.—Vitamins D bring about the absorption and utilization of both calcium and phosphorus in the body. Its deficiency thus interferes with the normal calcification of bones causing rickets and osteomalacia. An adequate intake is also necessary for the normal development of the teeth. The International Unit (I.U.) of vitamin D is defined as the vitamin activity of 0.025 γ of pure crystalline vitamin D₂ (calciferol) dissolved in one milligram of olive oil. A layer of freshly laid down deposit of calcium phosphate is formed when rachitic rats are given the antirachitic treatment, and this can be histo-

logically examined after proper staining; this method is used in the biological assay of this vitamin. For infants, a daily dose of 200-1200 I.U., for rickety children 1500-5000 I.U., and for adults 500-1000 I.U. are recommended.

Methods of Assay

Biological

(1) *Line Test Method*.—This is a curative method. Groups of young rats are fed on a rachitic diet when the animals develop rickets. Three groups are given varying quantities of the standard and another three groups of animals are given approximately equal quantities of the unknown. The tibia or ulna is removed and cleaned from adhering tissue. A longitudinal median section is made and the section is immersed in a 2 per cent aqueous solution of silver nitrate for one minute in the presence of light. The newly formed calcified area is thereby blackened which is studied under a low power microscope. The results are compared with the standards.

(2) *Bone Ash Method*.—This is one of the most accurate methods for the assay of vitamin D. Groups of young rats are fed on a rachitogenic diet. Four groups are given varying quantities of the standard vitamin D from the beginning of the experiment and another four groups varying quantities of the unknown. One group is kept as a control. After a period of 40 days, the femur is removed, freed from adhering tissues and from fat by extraction with alcohol and ether and the percentage of bone ash is determined. The results obtained with the unknown are compared with the standard.

(3) *X-ray Method*.—Young rats are fed on a rachitogenic diet and the degree of rickets determined by taking X-ray photographs of the bones. The rats are divided into two groups, one group getting daily doses of the standard preparation and the other group receiving the sample to be tested. The progress of the cure is followed by taking the X-ray pictures periodically and comparing the pictures of those getting the standard and those getting the unknown.

Chemical or Physical.—No chemical method has been developed which is specific enough for the assay of this vitamin in biological materials. The colour reaction with antimony trichloride is, however, useful in assessing the potency of pure vitamin D preparations. A saturated solution of antimony trichloride in chloroform is added to solution of the vitamin in chloroform and a yellow colour is developed with an absorption maximum at 500 m μ . The intensity of this colour can be determined spectroscopically.

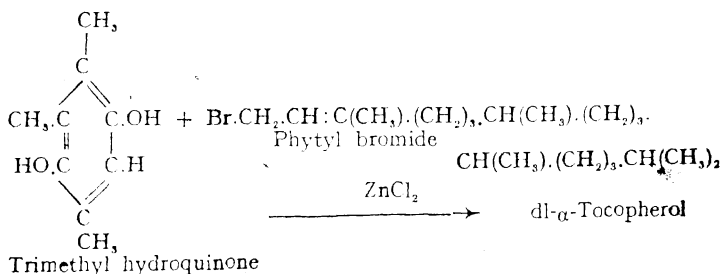
Vitamin E, Tocopherols (Gk. *tokos*—childbirth, *phero*—to bear).

This vitamin is also known as 'antisterility', 'reproduction', or 'fertility' vitamin. Three different compounds have been isolated having the physiological effects of vitamin E, and these are known as α -, β -, and γ -tocopherols.

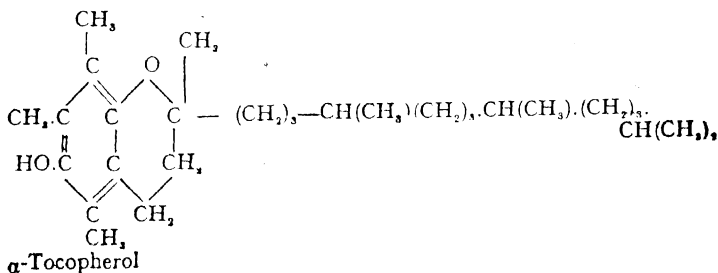
Occurrence.—The tocopherols occur predominantly in plant materials, and animal tissues contain only small amounts. The best natural source is the oil extracted from the germs of certain seed grains, such as wheat germ oil, rice germ oil, and cotton seed oil. Lettuce, alfalfa, etc., contain considerable amounts, while fruits contain only small amounts. Fish liver oils, which are rich in vitamins A and D, are poor in vitamin E. The relative proportions of α -, β -, and γ -tocopherols occurring in natural materials vary with the source.

Preparation.—It occurs in the unsaponifiable fraction of the oil, which is separated by a solvent such as ether from the saponified product and purified by distillation in high vacuum. The fraction distilling between 200° — 250° at 0.1 mm. contains the vitamin. The product is converted into an ester, e.g., allophanate (ester of allophanic acid, $\text{H}_2\text{N}.\text{CO}.\text{NH}.\text{COOH}$) which is purified by crystallization and the vitamin is regenerated from the ester.

Synthesis



Constitution, Physical and Chemical Properties.



The formulæ for β - and γ -tocopherols are similar. The α -, and β - and γ -tocopherols are oily liquids which have not as yet been obtained crystalline. Certain esters, such as the allopphanates, 3:5-dinitrobenzoates, etc., are crystalline. The tocopherols show characteristic absorption bands in the ultra-violet with a maximum at 295 m μ . In the absence of oxygen they are fairly stable to heat. They are not affected by dilute acids but alkalis decompose them slowly. They are sensitive to oxidation which destroys their biological activity. They are insoluble in water but soluble in absolute alcohol and in all ordinary fat solvents. They are fairly stable to visible light but are rapidly destroyed by ultra-violet light.

Physiological Action and Requirement.—This vitamin is essential for normal development of the foetus, and its deficiency causes male rats to become permanently infertile and in female rats there is no reproduction, the embryos being resorbed. Similar results have been reported in cows and sheep and the administration of vitamin E preparations has been found to be beneficial in habitual abortion in women.

Methods of Assay

Biological.—This may be carried out by putting female rats on a vitamin-E-deficient diet and putting others on the same diet but supplemented by graded doses of the unknown. The performance of the latter is compared with that produced by giving the pure vitamin or wheat germ oil, the criterion being the proof of the occurrence of gestation and resorption of the foetuses.

Chemical or Physical.—The method is based on the reduction of ferric chloride by vitamin E in alcoholic solution, and the production of a characteristic red colour of ferrous dipyrvidyl on the addition of α : α' -dipyridyl. Vitamin E is extracted with petroleum ether and the oily residue is saponified with 2N methyl alcoholic potash for about 10 minutes. The unsaponifiable fraction containing the vitamin is extracted with petroleum ether and the solvent removed. The residue is taken up in absolute alcohol and treated with ferric chloride and a solution of α : α' -dipyridyl. The red colour is compared in a colorimeter against the colour produced from the pure vitamin.

Vitamin K, Phylloquinone

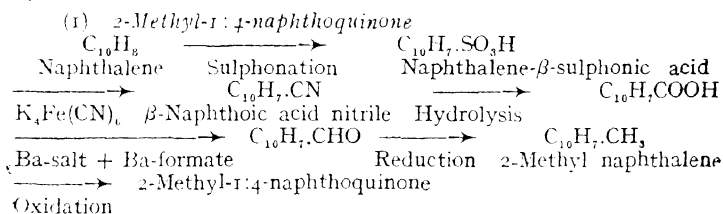
It is also known as 'coagulation vitamin,' 'prothrombin factor', or 'antihæmorrhagic vitamin'. The existence of two naturally occurring vitamins of high activity, viz., vitamin K₁

and vitamin K₂, has been proved with certainty. It is of interest to note that 2-methyl-1:4-naphthaquinone, a synthetic compound having the same ring structure as found in vitamins K₁ and K₂, possesses 3.3 times the activity of vitamin K₁ and about 5 times the activity of vitamin K₂.

Occurrence.—The best sources of vitamin K₁ are alfalfa, spinach, cabbage, kale, cauliflower and chestnut. It is also present in tomatoes, hempseed and soya beans. Fruits and cereals are very poor sources. Vitamin K₂ occurs in most bacteria, and putrefied animal materials contain high amounts due to bacterial growth. Hog liver is a rich animal source of vitamin K.

Preparation.—The dry material (alfalfa or putrid sardine meal) is extracted with petroleum ether and the extract is freed from some impurities with the help of adsorbing materials. The residual oil is distilled in high vacuum and the fraction distilling between 120° and 140° (at 10⁻⁴ mm. Hg) is collected. The individual components are then separated by using the chromatographic adsorption technique.

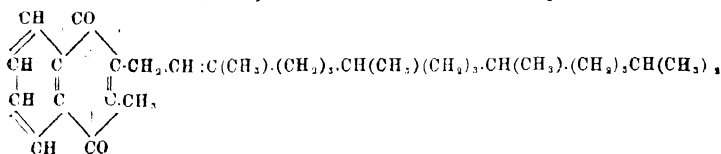
Synthesis :



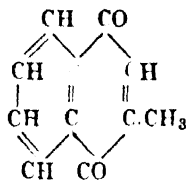
(2) *Vitamin K₁*:—

Mono sodium salt of 2-methyl-1:4-naphthohydroquinone + Phytol
bromide \longrightarrow Vitamin K₁

Constitution, Physical and Chemical Properties.



Vitamin K₁ (2-methyl-3 phytyl-1:4-naphthoquinone)



2-Methyl-1:4-naphthoquinone

Vitamin K_2 is 2-methyl-3-difarnesyl-1:4-naphthoquinone. The vitamins K are soluble in most of the common organic solvents such as ether, petroleum ether, acetone, hexane, etc., but is insoluble in water. They are stable to heat but are very sensitive to alkali and to light. Vitamin K_1 is a yellow oil which freezes at -20° ; vitamin K_2 is a yellow crystalline solid, m.p. 53.5° — 54.5° .

Physiological Action and Requirement.—Vitamin K is necessary for the formation of an enzyme (prothrombin) which plays a part in blood clotting, and in a number of conditions when there is a hæmorrhagic tendency, e.g., in obstructive jaundice, when vitamin K administration is valuable. It has, however, no effect in hæmophilia and thrombopenia. Vitamin K was discovered by Dam, after whom the unit known as the Dam Unit was named. One milligram of vitamin K_1 = 1000 Thayer-Doisy Units = 10,000 Dam Units. One gram of dried alfalfa hay contains about 300 Dam Units. One Dam Unit per gram of body weight with one gram of bile salts is the dose suggested per day to restore the prothrombin level to normal within 1-2 days.

Methods of Assay

Biological.—Different groups of chicks are fed with vitamin K deficient diet and the clotting time of their blood is determined occasionally. They are then fed with the sample and the regeneration of the normal clotting time is compared with those getting known quantities of vitamin K.

Chemical or Physical

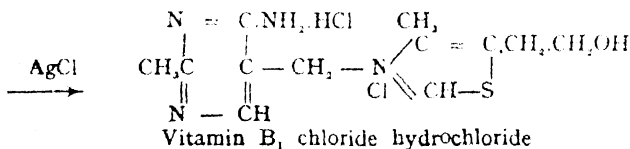
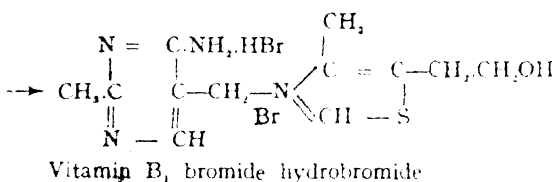
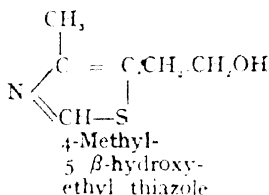
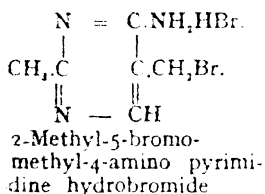
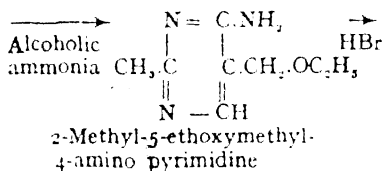
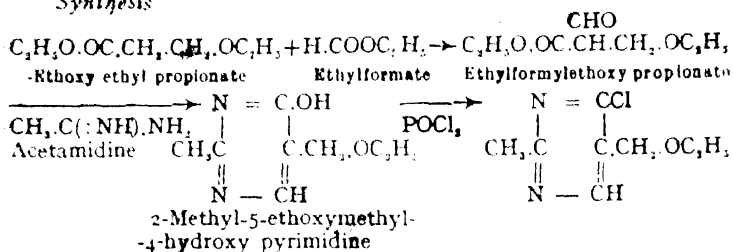
The natural vitamin K give a purple-blue colour with sodium ethylate or sodium methylate. The sensitiveness of this colour reaction can be improved by preparing the 2:4-dinitrophenyl hydrazones of the vitamins. An alcoholic solution of the vitamin, which is a quinone, is treated with a solution of 2:4-dinitrophenyl hydrazine in

dilute HCl and gently warmed. The bluish green colour developed by the addition of sodium methylate or the green colour formed with ammonia and amyl alcohol is fairly stable and can be utilized for the chemical assay of this vitamin.

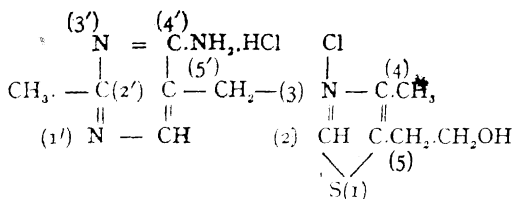
Vitamin B₁, Aneurin, Thiamin, Antiberiberi Vitamin

Occurrence.—This is widely distributed both in plant and animal tissues. The following figures in brackets represent micrograms per 100 grams of substance: Whole grains, such as wheat (500—660), unpolished rice (240—300), or oats (345—770), maize (200—300), pearl millet or *bazra* (330—380), kaffir corn or *jowar* (270—490), baker's yeast (1000—3000), brewer's yeast (5000—8000), food yeast (*Torula utilis*) (2200—4100), marmite (1250), dry peas (300—620), *chho'a da'* (300—450), *mung dal* (400—800), *masur dal* (300—600), *arhar dal* (210—700), soya beans (300—900), almonds (120—330), ground nuts (740—900), sesame (*til*) seeds (1,000—1,100), cashew nuts (600—800), etc., form very good sources. Yeast, wheat germ, and rice polishings are three of the richest sources. Vegetables such as green peas (270—495), spinach (95—155), tomatoes (70—115), or fruits such as bananas (50—100), orange (75—145), coconut (70—150) form fair sources, and animal tissues such as mutton (200—300), kidney (400—500), liver (300—420), and egg yolk (350—440) are also fair sources. In animal tissues this vitamin is present partly as an ester of phosphoric acid and partly in the free state.

Preparation.—The material (rice polishings or yeast) is extracted with cold or hot water adjusted to pH of 4.5. The vitamin is adsorbed from the aqueous extract by activated fuller's earth or by activated charcoal at pH 6.5. The vitamin is eluted from the activated charcoal with a dilute mineral acid or from the fuller's earth with a dilute alkali. The vitamin is finally isolated as the hydrochloride by an elaborate procedure involving fractional precipitation and crystallization. 450 pounds of rice polishings or 2000 pounds of fresh yeast yield only about one gram of the pure vitamin at a cost of about 1000 rupees. The synthetic compound costs only about a rupee per gram.

Synthesis

Constitution, Physical and Chemical Properties.—The molecule of vitamin B₁ contains a *pyrimidine* nucleus and a *thiazole* nucleus, and has the following structure:



It is also known as 'thiamin hydrochloride' or 'aneurine chloride hydrochloride', and its structure corresponds to 3-(4'-amino-2'-methylpyrimidyl-5'-methyl)-4-methyl-5 β -hydroxyethyl thiazolium chloride hydrochloride.

The vitamin hydrochloride crystallizes from aqueous alcohol in colourless needles or plates, m.p. 248° - 250° . The crystals and pure solutions have a faint bran-like odour. It is soluble in water (1g. in 1 c.c.) and in 95 per cent alcohol (1g. in 100 c.c.) but is almost insoluble in ether, chloroform or benzene. It is optically inactive. It is quite stable in aqueous acid solutions. In neutral medium it is slowly decomposed, the destruction being very rapid in alkaline solutions. In a solution of sodium sulphite, the molecule undergoes a cleavage into two portions. It is very sensitive to oxidizing agents.

Physiological Action and Requirement.—Vitamin B₁ promotes growth and prevents beri-beri and polyneuritis. It also promotes the recovery of appetite and maintains the normal motility of the digestive tract. The polyneuritis with paralysis and heart failure which develops in birds in its deficiency is probably a functional disturbance due to the accumulation of pyruvic acid, an intermediate in the oxidation of carbohydrates, and thiamin catalyzes the transformation of pyruvic acid and thus helps the carbohydrate metabolism.

The International Unit of vitamin B₁ is 3 micrograms of the pure substance. The requirements for different ages are: children 1-12 years, 0.4 to 1.2 milligrams; boys and girls 12-20 years, 1.2 to 2.0 mgm.; adults 1.2 to 2.3 mgm. (1 mgm. = 333 I.U.).

Methods of Assay

Biological.—Young rats are fed upon a diet which contains all the essentials for growth except vitamin B₁. The growth continues

for 2 or 3 weeks and then ceases. When the weight is stationary, the rats are divided into two groups. One group receives the material to be examined and the other group gets a standard preparation of the vitamin. From the average increase in weight of the rats in each group, the activity of the material can be ascertained.

Chemical or Physical

(1) *Thiochrome Method*.—Vitamin B_1 is extracted from the finely powdered or minced food material (2.5 g. of cereals, pulses or nuts, 10 g. of vegetables, fruits or animal tissues) by mixing the material with 50 c.c. of acetate buffer (pH 5; made by mixing 1 part of N/10 Na-acetate with 2 parts of N/10 acetic acid) and digesting with papain (0.5 g. to liberate vitamin B_1 from its combination with proteins) and Taka-diastase (0.5 g. to liberate vitamin B_1 from its combination with pyrophosphoric acid by the phosphatase present in Taka-diastase) for about 6 hours at 37°C. (N.B. In the case of cereals, the use of papain and Taka-diastase may be omitted and the material extracted with one per cent HCl, 50 c.c. for 5 g.). The extract is centrifuged and an aliquot part (1-2 c.c.) of the clear centrifugate is oxidized with potassium ferricyanide (0.2 c.c. of 2 per cent) in an alkaline medium (3 c.c. of N NaOH). 15 c.c. of isobutyl or isoamyl alcohol are added and the mixture shaken for one minute to extract the thiochrome formed. A blank estimation is carried out in the same manner without only the addition of potassium ferricyanide. 10 c.c. of each of the alcoholic extracts are taken in two test tubes and clarified by the addition of one c.c. of rectified spirit. (Standard: 10 micrograms of pure vitamin B_1 are oxidized to thiochrome in the above manner and extracted with 20 c.c. of isobutyl or isoamyl alcohol; one c.c. of the extract represents 0.5 microgram of vitamin B_1). The two test tubes containing the blank and the oxidized samples are exposed side by side to Ultra-violet rays from a mercury vapour lamp filtered through a Wood's glass filter. The oxidized sample will show a deeper fluorescence than the blank if there is any vitamin B_1 . Small quantities of the standard thiochrome extract are gradually added from a microburette to the blank and both the tubes are exposed to U.V. rays after each addition till the intensity of fluorescence is the same in both the tubes. From the amount of standard solution required, one can easily calculate the quantity of vitamin B_1 present in the material assayed.

(2) *Colorimetric Method*.—Diazotized p-aminoacetophenone gives with vitamin B_1 a red azo dye which can be extracted with an organic solvent such as xylene. The colour can be matched against a standard using pure vitamin B_1 . The reaction is very sensitive and 5 micrograms of the vitamin can be easily estimated.

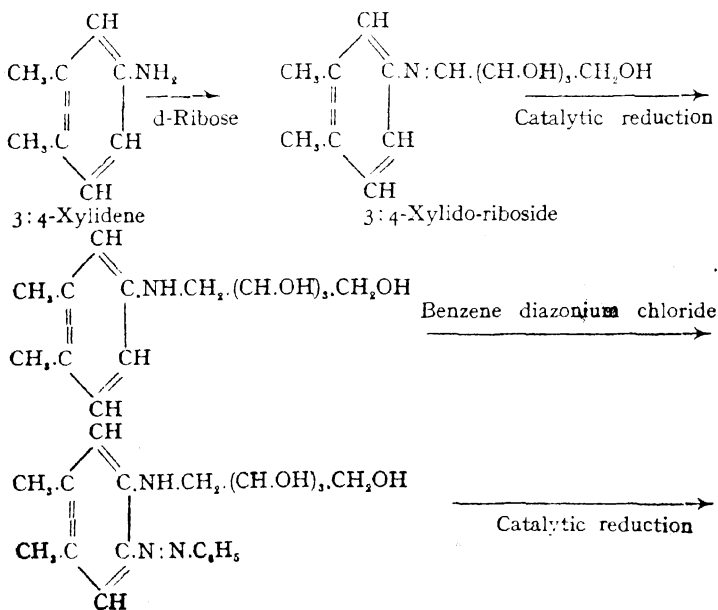
Riboflavin, Lactoflavin, Vitamin G, Ovocflavin, Renoflavin, Hepatoflavin, (originally, Vitamin B_2)

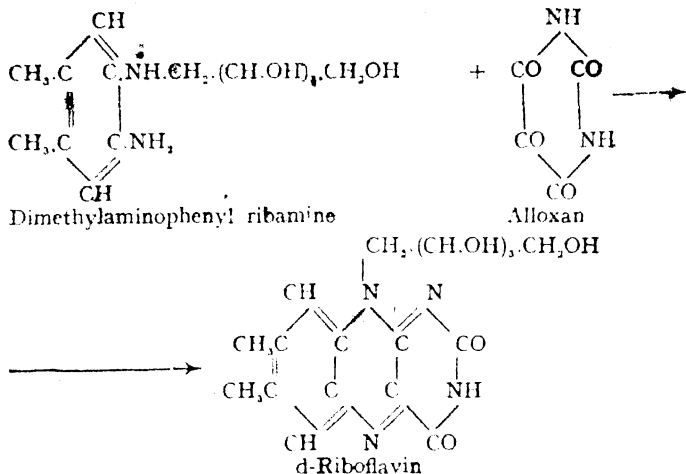
Occurrence.—This is widely distributed both in plant and animal tissues. The following figures in brackets repre-

sent micrograms per 100 grams of substance. Mammalian liver (1,800—4,700), kidney (1,700—2,200), milk (195—240), eggs (280—420), wheat germ (600—800), unpolished rice (115), whole wheat (100—220), pulses or dals (250—330), spinach (250—400), *notey sag* (55), *dhoney-sag* (coriander) (98), *lettuce* (100—240), *kalmi-sag* (149), *kumra-sag* (158), onion (50), brewer's yeast (2,500—4,700), baker's yeast (2,000—4,000), food yeast (5,500—7,000), and marmite (8,920).

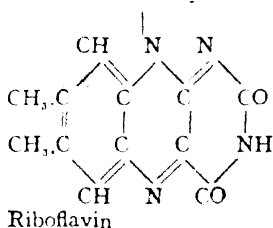
Preparation.—The material is extracted with an aqueous acid solution or with a mixture of water and alcohol. The vitamin is adsorbed from the extract in acid solution with fuller's earth and eluted from the adsorbing material with the help of dilute alkali. The riboflavin is purified further by means of silver salts. 200 pounds of dried egg-white (about 33,000 eggs) gave only 100 milligrams of pure riboflavin. The synthetic product now costs about 5 rupees per gram.

Synthesis





Constitution, Physical and Chemical Properties.
 $\text{CH}_2\text{CHOHCHOHCHOHCH}_2\text{OH}$



6:7-Dimethyl-9-(d-1'-ribityl)-isoalloxazine

Riboflavin crystallizes in orange-yellow needles, m.p. 282° , and is bitter to taste. It is slightly soluble in water (12 mgms. in 100 c.c. at 27.5°), slightly soluble in alcohol, and insoluble in the usual fat solvents. It is very soluble in alkalis. It is stable to heat in neutral or acid medium but is destroyed by alkali, slowly in the cold and more rapidly at elevated temperatures. It is decomposed by visible or ultra-violet light. Its aqueous solutions are greenish yellow in colour and show yellow green fluorescence in ultra-violet light, with a maximum at $565 \text{ m}\mu$. On reduction with sodium bisulphite or with stannous chloride, it loses its yellow colour and it can be reoxidized to the coloured form by oxygen or mild oxidizing agents.

Physiological Action and Requirement.—Riboflavin takes part in the oxidation processes of the cell, and its phosphoric acid ester combined with a protein is probably identical with Warburg's 'yellow enzyme'. Its deficiency causes macerated areas in each angle of the mouth, cracking of the lips, sensitiveness of the eyes to light, scrotal dermatitis and superficial keratitis. It is essential for growth and for normal nutrition at all ages.

The Bourquin-Sherman Unit of this vitamin, formerly known as vitamin G, is 2.5 micrograms of riboflavin, *i.e.*, the amount that supports a growth rate of 3 g. a week in rats on a standardized diet. The daily requirement for human beings ranges from 0.6 mgm. for a child of 1 year to 3.0 mgm. for an adult.

Methods of Assay

Biological.—(1) It may be assayed with the help of growing rats subsisting on a riboflavin-deficient diet, supplemented by the pure vitamin for one group and by graded doses of the unknown for another group.

(2) It is also assayed by studying the stimulating effect of this vitamin upon the growth of certain bacteria (*e.g.*, *Lactobacillus casei*) in synthetic media, the growth being estimated either by the turbidity produced or by the lactic acid formed.

Chemical or Physical.—The riboflavin is extracted from the biological material and purified to some extent by adsorption and elution. (1) The fluorescence of the riboflavin itself may be determined and compared with that of pure riboflavin, or (2) the fluorescence of the lumiflavin formed by the irradiation of the riboflavin in alkaline medium is determined and compared with that obtained with pure riboflavin.

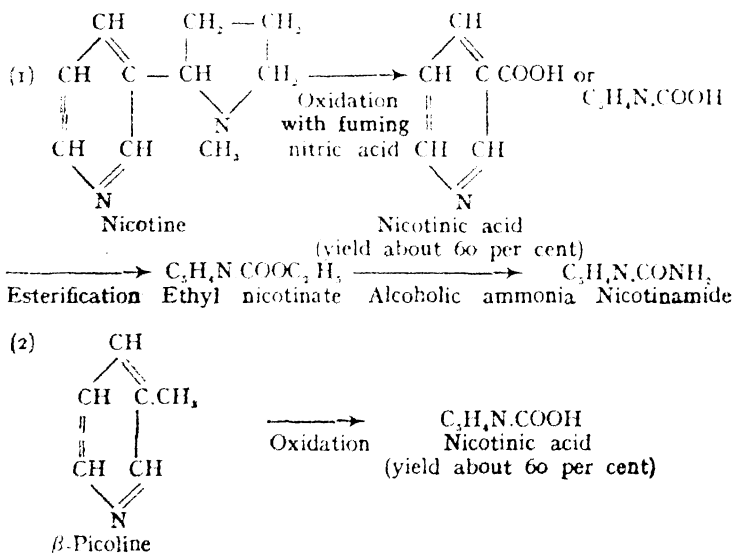
Nicotinic Acid (Niacin) and **Nicotinamide** (Niacinamide), *Pellagra-preventive Vitamin* (P-P Factor).

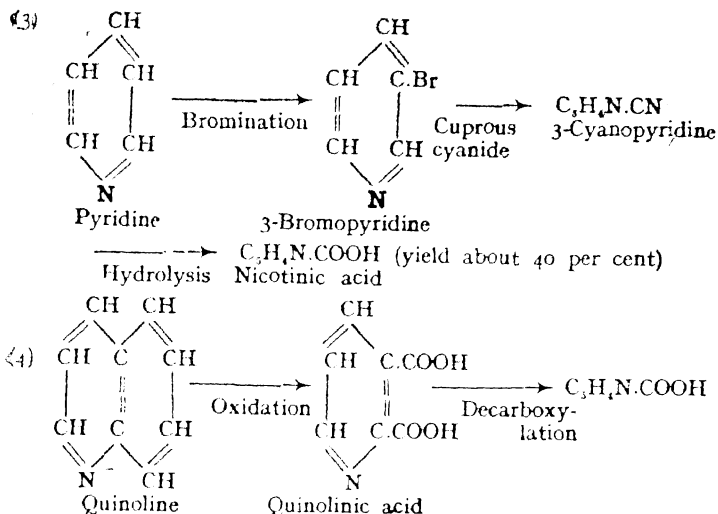
Occurrence.—The acid and its amide occur in small amounts in plant and animal tissues. The following figures in brackets represent milligrams of *nicotinic acid* per 100 grams of food material: whole barley (3.0—4.5), American barley (8—14), maize, dry (1.4—2.0), unpolished rice (2.8—4.0), raw milled rice (0.9—1.2), whole wheat (4.6—5.0), refined wheat flour (0.8—1.0), *chhola dal* (2.5—2.7), *mung dal* (1.7), *musur dal* (1.5), peas, dry

(1.3), groundnut (13.4—14.8), *til* seeds (4.4), *data-sag* (Amaranth) (0.9), cabbage (0.4), *kalmi-sag* (Ipomoea) (0.8), *kumra* leaves (0.9), carrot (0.4), potato (1.2), sweet potato (0.8—1.2), brinjal (0.8), lime juice (0.1), orange juice (0.3), tomato (0.4), cow's milk (0.1), buffalo milk (0.1), mutton (4.1—8.0), goat liver (12.5—16.0), fish (0.6—3.9), dried yeast (15—50), tea (6.1), coffee (13.2), cocoa (1.6).

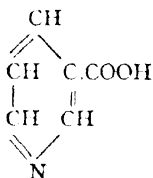
Preparation.—Nicotinic acid and nicotinamide can be isolated from the material by extraction with water, adsorption from the extract with activated charcoal and elution of the adsorbing material with a mixture of pyridine and alcohol. The residue from the extract is shaken out with chloroform, which dissolves out the amide, and after removal of the chloroform the product is distilled fractionally under high vacuum. Nicotinamide distils at 150° — 160° at 5×10^{-4} mm. Hg. The distillate is crystallized from benzene. The free acid which remains behind after extraction with chloroform can be precipitated as a copper salt from which it is regenerated and purified.

Synthesis of Nicotinic Acid and Nicotinamide

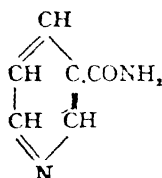




Constitution, Physical and Chemical Properties



Nicotinic acid
(Pyridine-*p*-carboxylic acid)



Nicotinamide
(Amide of nicotinic acid)

Nicotinic acid crystallizes from water or alcohol in colourless needles, m.p. 235.5° – 236.5° . It sublimes without decomposition. It has the properties of an acid as well as of a base, forming salts with alkalis and acids. It is soluble in water (about 2.5 per cent) and alcohol and is sparingly so in fat solvents.

Nicotinamide crystallizes from benzene in colourless needles, m.p. 129° . It is hydrolyzed by strong mineral acids or by alkalis to nicotinic acid. It is soluble in water, alcohol, chloroform and benzene and is fairly stable to heat.

Physiological Action and Requirement.—As the name 'pellagra-preventive' factor indicates, the deficiency of nico-

tinic acid or its amide causes pellagra, a kind of dermatitis accompanied by inflammation of the tongue, and also some digestive and nervous disturbances.

The usual dose for the treatment of pellagra is stated to be 300—500 milligrams of the acid or its amide. The normal daily requirement for an adult is about 15—25 milligrams, for children 1-12 years, 4-12 mgms., and for boys and girls over 12 years, 12-20 mgms.

Methods of Assay

Micro-Biological.—The amount of lactic acid produced by *Lactobacillus arabinosus* is estimated, the quantity of lactic acid formed being proportional to the amount of nicotinic acid or nicotinamide present when the bacillus is supplied with adequate quantities of all necessary growth factors.

Chemical or Physical.—A weighed amount (10-20 g) of the finely ground material is extracted with hot water, the protein, etc., precipitated by lead acetate and the excess of Pb removed by sulphuric acid. Conc. HCl is added to make about 5 per cent solution, heated on water bath for 30 minutes to hydrolyze the nicotinamide, the solution decolorized with freshly precipitated $Zn(OH)_2$, the pH adjusted to 7 and made up to a known volume. An aliquot part is treated with cyanogen bromide-aniline reagent and the yellow colour developed is compared against a standard solution of nicotinic acid treated similarly (Swaminathan).

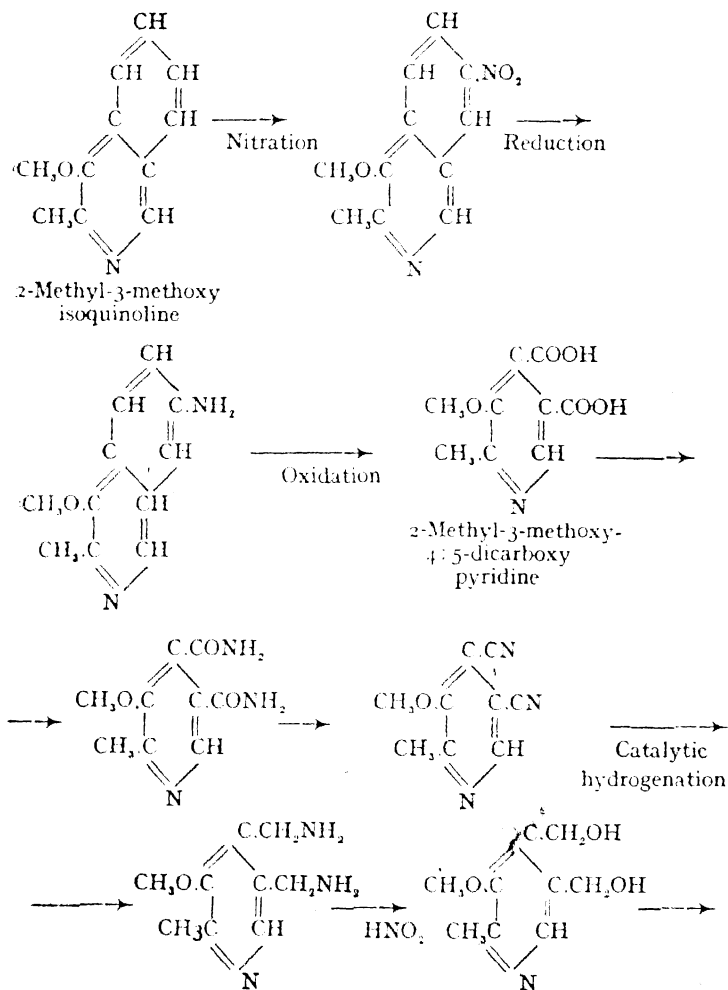
Vitamin B₆, Pyridoxin, Adermin

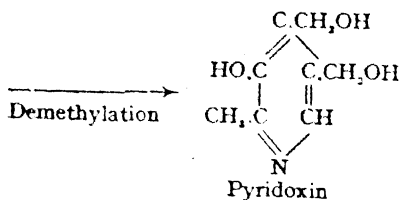
Occurrence.—It is widely distributed both in the plant and animal kingdoms, yeast, rice polishings and wheat germ being the richest sources. The following figures in brackets represent micrograms of vitamin B₆ (pyridoxin) per gram of food material: rice, raw, milled (2.3—3.3), whole wheat (4.2—6.4), white flour (1.2—2.5), Bengal gram (10—11), green gram (10—11), soya bean (8.0—12.0), black gram (9.9—11.3), cabbage (2.6—2.9), carrot (1.9—2.2), brinjal (1.9), plantain, raw (1.4), French bean (0.96), potato (1.6—2.7), mango, ripe (2.8), orange (1.75), goat liver (3.7—6.1), goat muscle (3.0—4.6) fish (2.1), egg, hen's (0.7), cow's milk (0.5—1.2), ground nut (7.3), dried yeast (39—52).

Preparation.—The method of isolation from natural sources is similar to that of vitamin B₁. The vitamin is extracted with

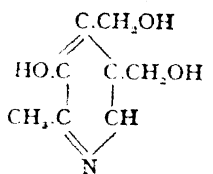
acidulated water, adsorbed in fuller's earth and eluted with barium hydroxide. It is precipitated with phosphotungstic acid and isolated as hydrochloride after removal of interfering substances by treatment with different reagents. The free base is prepared from the chloride by treatment with a silver salt.

Synthesis





Constitution, Physical and Chemical Properties



Pyridoxin

2-Methyl-3-hydroxy-
4:5-di- (hydroxy-
methyl) -Pyridine

The free base is a colourless crystalline powder, m.p. 160° . It has a slightly bitter taste and is readily soluble in water, acetone and alcohol, but sparingly so in ether or chloroform. It is stable to heat and to acids or cold alkalis but is destroyed by light.

Physiological Action and Requirement.—In the rat its deficiency produces pellagra-like conditions. In humans, extreme nervousness, irritability, abdominal pain and weakness are reported as symptoms of the deficiency of this vitamin. The knowledge about the normal human requirement is lacking.

Methods of Assay

Biological.—It can be assayed by feeding vitamin B₆-deficient diet to rats and causing pellagra, and curing or preventing the disease by giving graded doses of the unknown.

Chemical or Physical

(1) This vitamin gives an orange-yellow azo dye with diazotized sulphanilic acid. The reaction is very sensitive and as little as 5 micrograms can be estimated by this method.

(2) This vitamin gives a blue indophenol colour when mixed with 2:6-dichloroquinone chloroimide with an absorption maximum at 660 m μ . This reaction is very sensitive and 20 to 50 micrograms can be easily estimated in a colorimeter or spectrophotometer (Phenolindophenol Test).

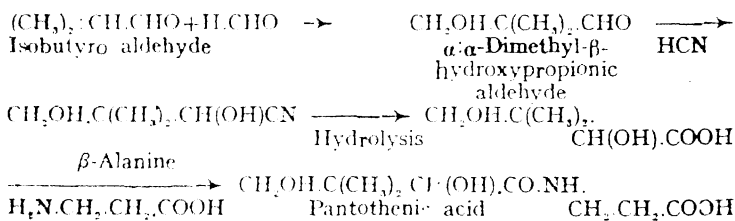
Pantothenic Acid

Occurrence.—It occurs in all types of animal and plant tissues. The liver and kidney are the richest animal sources, followed by heart, spleen, brain, pancreas, etc. Yeast and

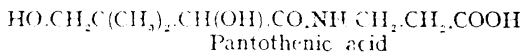
rice bran are rich sources. Whole cereals, pulses, and nuts are good sources but vegetables and fruits contain little.

Preparation.—From the liver it has been isolated by extraction with water after autolysis, adsorption on activated charcoal at pH 3.6, and elution from the charcoal with ammonia. It is then converted into the brucine salt and the salt is extracted with chloroform. It is finally converted into the calcium salt which is purified by recrystallization. 250 kilograms of liver yielded about 3 g. of crude pantothenic acid

Synthesis



Constitution, Physical and Chemical Properties



[α : γ -Dihydroxy- β : β -dimethyl butyryl- β -aminopropionic acid]

It shows both acid and basic properties. It is readily soluble in water, ethyl acetate and glacial acetic acid, but sparingly soluble in ether and amyl alcohol. It is sensitive towards hot acid or alkali, but is stable in neutral medium. When pure it is a pale yellow viscous oil. It has a sp. rotation $[\alpha]_D^{25} = +37.5^\circ$. The calcium salt, which is microcrystalline, has the sp. rotation $[\alpha]_D^{26} = +24.3^\circ$.

Physiological Action and Requirement.—The role in human nutrition is not known. It is stated to be the chick-pellagra factor, necessary for the growth of the rat, and for the prevention of the greying of the hair of black rats.

Methods of Assay

Biological.—(1) It is assayed by the use of chicks which develop pellagra-like symptoms on a pantothenic acid deficient diet.

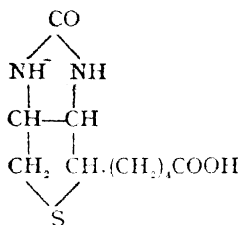
(2) It is also assayed by studying the stimulating effect of this vitamin upon the growth of certain bacteria on synthetic media.

Vitamin H, Biotin, $C_{10}H_{16}O_3N_2S$

Occurrence.—It is found in small amounts in all plant and animal tissues, and its distribution is similar to that of pantothenic acid.

Preparation.—The material (liver or yeast) is extracted with acidulated water after liberating the vitamin by proteolytic digestion. Some interfering substances are removed by lead acetate and the vitamin is precipitated by phosphotungstic acid. It is further purified by electrodialysis or by distillation in high vacuum. 250 kilograms of dried Chinese egg yolk gave only 1.1 milligrams of pure biotin.

Synthesis.—It has been recently synthesized in the laboratory.

Constitution, Physical and Chemical Properties

The pure vitamin melts at 230° — 232° and the methyl ester melts at 166° — 167° . It shows a sp. rotation $[\alpha]_D^{22} = +92^\circ$ in N/10 NaOH solution. The pure vitamin is easily soluble in chloroform or ether. It is stable to heat and is

easily adsorbed on charcoal from aqueous acid solutions.

Physiological Action and Requirement.—Its role in human nutrition is not known. It is stated to prevent the so-called 'egg white injury', and it is essential for the growth of yeast and certain bacteria.

Methods of Assay

Biological.—It can be assayed by studying its effect on the growth of yeast, or by the amount of lactic acid produced by the growth of *Lactobacillus arabinosus* in a medium containing all the other growth essentials.

Chemical or Physical.—Not known.

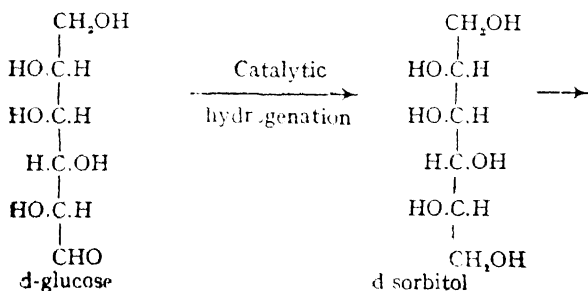
Vitamin C, Ascorbic Acid, Cevitamic Acid, Hexuronic Acid

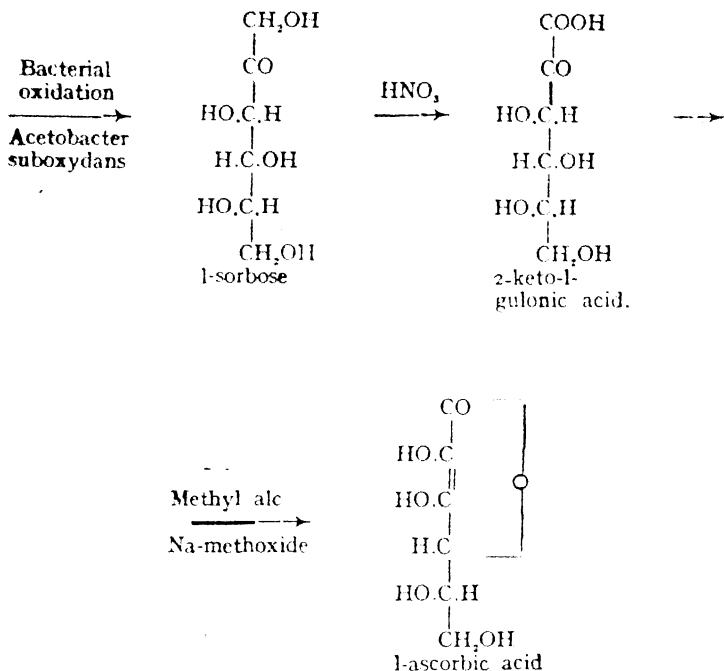
Occurrence. It is widely distributed in the animal and plant kingdoms. Fresh fruits and green vegetables are rich

sources (the figures in brackets representing milligrams per 100 grams), *e.g.*, lemons (39—60), Indian guava (100—290), South African guava (100—700), orange (38—68), mango green (3), mango ripe (13—69), banana (7—8), Indian gooseberry or *amloki* or *amla*, (*Phyllanthus emblica* Willd.) (300—700), tomato (22—40), cabbage (40—124), green peas (9—30), turnip (17—43), papaya (33—55), *notey-sag* (42—173), and *kalmi-sag* (137), radish (16—43), sweet potato (22—24), potato (12—36), onions (8—11), jack fruit, *kanthal* (10—16), pomegranate (10—16), pomelo (20—62), *pudina* (123), *neem* leaves (129), *sajina* leaves (146—230). Dried cereals, pulses and nuts are devoid of this vitamin, but germinated pulses contain fair amounts (10—20). Rose-hips and paprika are stated to be very rich sources. In the animal kingdom, the concentration is highest in the glandular tissues, such as adrenals, pituitary, corpus luteum, etc., and are lowest in blood and muscular tissues.

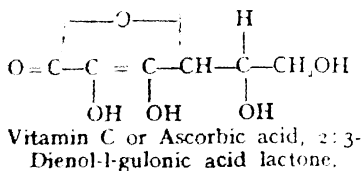
Preparation.—The freshly pressed juice (*e.g.*, lemon) is treated with barium acetate or neutral lead acetate and filtered. The vitamin is then precipitated as the lead salt by adjusting the pH to 7.6 with ammonia. The precipitate is decomposed with dilute sulphuric acid at pH 2, some colouring matter removed by extraction with *n*-butyl alcohol, and the filtrate concentrated in vacuo below 60°. The vitamin is precipitated with acetone or ether. It is purified by crystallization from methyl alcohol. Hungarian pepper yielded 20—25 per cent of ascorbic acid.

Synthesis



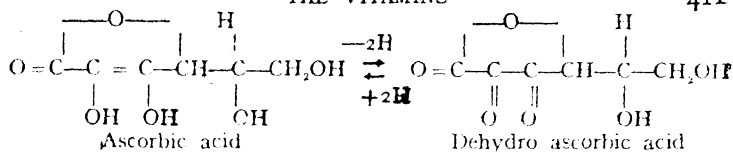


Constitution, Physical and Chemical Properties



Vitamin C crystallizes in colourless plates, m.p. 190° — 192° . It is freely soluble in water but sparingly so in alcohol or acetone, and is insoluble in ether and petroleum ether. It shows a sp. rotation $[\alpha]_D^{20} = +23^\circ$

(water), and $[\alpha]_D^{25} = +48^\circ$ (methyl alcohol). The pure crystalline solid is quite stable but in solution it is rapidly oxidized by the oxygen of air, especially in the presence of heavy metals such as Cu or Ag, to the reversibly oxidized form known as *dehydro ascorbic acid*:



It thus shows the characteristic strong reducing action and its chemical assay depends upon this property. The oxidation is more rapid in alkaline than in acid solutions, and it is not auto-oxidized within the normal pH range of plant and animal tissues.

Ascorbic acid, a typical dienol, responds to the dinitro benzene test (see p. 203). The violet colour is produced immediately with the cold reagent. No warming is necessary.

Physiological Action and Requirement.—It is necessary for the formation of intercellular material which cements together the endothelial cells, and the loss of the cementing material lowers the strength of the walls of the blood vessels giving rise to hæmorrhage, the condition being known as *scurvy*. It is also said to be an essential factor for normal tooth formation and its deficiency lowers the resistance of the body to infection.

The International Unit of vitamin C is defined as the specific anti-scorbutic activity contained in 0.05 milligram of the pure substance. The normal requirement for children and adults is stated to be 28—100 milligrams of the pure substance per day.

Methods of Assay

Biological—

(1) *From Changes in the Histological Structure of the Teeth.*—One group of guinea-pigs is fed on diets deficient in vitamin C, and another group receives different doses of a standard preparation of vitamin C. After a fortnight they are killed and the lower jaw bones are removed and decalcified. Sections are cut of the root of the incisor and stained. The extent of disorganization of the structure is compared with those receiving the standard preparations.

(2) *From Growth.*—Guinea-pigs beginning to decline on a scorbutic diet are fed with graded doses of the unknown and their growth compared with that of positive controls.

Chemical or Physical

The material is extracted with a mixture of 10 per cent trichloroacetic acid and 10 per cent metaphosphoric acid. The acid extract is

then titrated with a standardized indicator solution of 2:6 dichloro-

phenol indophenol, $O = \text{C}_6\text{H}_4 = \text{N} - \text{C}_6\text{H}_4 - \text{OH}$, which

reduced to the colourless substance $\text{HO} - \text{C}_6\text{H}_3(\text{Cl})_2 - \text{NH} - \text{C}_6\text{H}_4 - \text{OH}$

The freshly prepared dye solution is standardized against pure ascorbic acid since the commercial dye may not be quite pure; 20 milligrams of the pure dye dissolved in 100 c.c. water is reduced by about 10 milligrams of pure ascorbic acid. In case pure ascorbic acid is **not** available, the dye can be standardized against a standard iodine solution, one c.c. of N/200 iodine being equivalent to 0.44 milligram of ascorbic acid. 20 c.c. of the dye solution is added to 5 grams of KI dissolved in 20 c.c. water, 10 c.c. of 20 per cent acetic acid is added and the liberated iodine is titrated against freshly prepared thiosulphate solution.

For urine, measure out 0.5 c.c. of a 0.05 per cent of the dye solution into a hard glass test tube, add 1.0 c.c. of glacial acetic acid, and run in the *fresh* urine from a small burette until the red colour is just discharged. The concentration of the acetic acid should be kept at about 10 per cent, and if the volume of the urine exceeds 10 c.c. the experiment should be repeated by taking more acetic acid. The titration should be completed within two minutes, but not faster than half a minute. Since 1 mgm. of the dye is equivalent to 0.5 mgm. of pure ascorbic acid, the percentage of the latter can be easily calculated. The addition of 1 vol. of acetic acid to 10 vols. of urine helps to preserve the urine for this estimation for about 4 hours.

Vitamin P, Citrin, Permeability vitamin

This vitamin occurs in citrus fruits such as lemon, orange, grape fruit, etc. Its chemical nature has not as yet been definitely established but highly potent concentrates prepared from the above sources are known to contain the glucosides hesperidin and eriodictin besides other unknown active components. The deficiency of this vitamin causes a decrease in capillary resistance resulting in a hæmorrhagic condition of the skin known as "nutritional purpura", not cured by vitamin C. The daily protective dose is stated to be 50 to 100 c.c. of orange juice.

PART V

TOXICOLOGICAL CHEMISTRY

CHAPTER XXXII

Introduction. Classification of Poisons. Selection of Materials. Preservatives. Routine Procedures for Chemical Analysis. Methods of Extraction.

Introduction

Toxicology is the science of poisons and implies the study of their origin, their properties, their action on the organism, the treatment of their harmful effects, and their detection and quantitative estimation. The subject is, therefore, divided into *medical* or clinical toxicology and *forensic* toxicology, the scope of the latter being the detection of criminal poisoning by chemical or other means. The term toxicology has been derived from the Greek word 'toxicon' which means poison, particularly those poisons with which the primitive man used to paint the arrow-heads (*toxa*—bow and arrow) to render them more effective in killing their enemies, men and animals.

It is very difficult to define the term 'poison'. A substance which is harmless and is necessary for maintaining health may act as a poison under certain conditions. Common salt, for instance, is a harmless substance but is known to have been successfully used in this country for getting rid of unwanted babies by putting it into their mouth immediately after they are born. On the other hand, all poisonous substances which are criminally used for homicidal or suicidal purposes are very efficient weapons in our armoury for curing diseases and saving life. In fact, it is the quantity of the substance or in other words its dosage that really accounts for its poisonous or therapeutic effects. It is stated that "there is no soluble substance known that does not possess toxic properties when given in sufficiently large doses." The detection of a poison and its quantitative estimation are, therefore, the essential features of forensic

toxicology, and toxicological chemistry is the basis of all such investigations in cases of poisoning, fatal or otherwise.

Classification of Poisons

Poisons are classified in various ways—the usual classification being based on the signs and symptoms they produce on the human system, e.g., irritants, corrosives, neurotics, spinal, cardiac, etc. For purposes of chemical analysis, however, the following classification is helpful to the toxicological chemist:—

1. Gaseous Poisons.
2. Corrosives.
3. Organic Poisons:
 - (a) Volatile Poisons (including volatile alkaloids).
 - (b) Non-volatile Poisons:
 - (i) Alkaloidal.
 - (ii) Non-alkaloidal.
4. Inorganic Poisons:
 - (a) Non-metallic Poisons.
 - (b) Metallic Poisons.

Selection of Materials for Chemical Analysis

The proper selection of materials for chemical analysis in cases of poisoning either fatal or non-fatal, is very important in chemicolegal investigations. The following materials should, as far as possible, be collected either by the medical officer who holds the post-mortem examination or by the investigating police officer who is placed in charge of such cases:—

1. *Organs or tissues forming the routes by which the poison is administered:* Stomach with its contents; rectum (in cattle poisoning by *Abrus*, *Calotropis*, etc.); vagina and uterus (in cases of criminal abortion by local application of the poison); skin and subcutaneous tissue (in cattle poisoning by *Abrus*, and in human poisoning by subcutaneous injection of the poison); lungs (in cases of poisoning by HCN , CHCl_3 , or other volatile poisons).

2. *Organs through which the poison is eliminated:* Kidneys and intestines.

3. *Organs or tissues in which the poison is retained after absorption in the system:* Liver and kidney (in all cases of poisoning); blood and brain (in cases of poisoning by alcohol and CHCl_3 and also by CO and other gaseous poisons); hair (in chronic poisoning by arsenic or lead); cerebro-spinal fluid (in alcohol poisoning).

4. *Excreta and other materials with which the poison is eliminated from the system:* Urine, fæces, vomited matter and stomach-washing with plain water (not with permanganate solution) in all cases of poisoning; urine, sweat and saliva (in cases of doping of race horses).

5. *Vessels in which the poison is suspected to have been powdered, dissolved or stored before its administration:* Bottles, cups, tumblers, pestle and mortar, sil and nora, hypodermic syringe with its needle, etc.

6. *Miscellaneous substances:* Remnants of food or any medicine suspected to have been taken or administered before the onset of symptoms of poisoning; pill- or powder-boxes and pieces of paper with which a medicine is wrapped and dispensed; bed sheets or garments soiled with vomited matter, urine, fæces, etc.

Selection of Preservatives for Preventing Decomposition of Materials for Chemical Analysis

Rectified spirit (90 per cent alcohol) is always used as a preservative for viscera, urine and other materials which are likely to decompose on keeping. It is, however, contraindicated in cases of poisoning by alcohol, phosphorus, paraldehyde, acetic acid, carbolic acid, or any other member of the phenol group. In such cases, a *saturated solution of common salt* (NaCl) is used. If the salt solution is not saturated, the putrefying bacteria, usually present in the viscera (intestines), continue to grow there and decompose the organic matter along with the poisons (particularly the alkaloids and glucosides) retained therein; and yeast and other fungi, if they happen to be present in the stomach, cause fermentation of carbohydrate foods especially during the hot months of the year, and produce ethyl alcohol and other fermentation products. A serious fallacy is thus introduced into the investigation which would, therefore,

result in the reporting of a non-alcoholic case as one of alcohol poisoning. It is, therefore, the duty of the analyst to see that the salt solution, a sample of which is always sent, according to Government orders, along with the viscera and other materials for analysis, is really a saturated solution (containing about 33 per cent of NaCl). It has been found by actual experiments by one of us (K.N.B.) that the food materials collected from the stomach during post mortem examination could easily be fermented even in the presence of 15 per cent salt solution simply by keeping on the laboratory table for about 48 hours. As the fluid portion of the stomach contents tends to dilute the salt solution considerably, and as a low concentration of salt favours the growth of fermenting organisms, it is imperative that a large excess of correctly prepared saturated salt solution is added to the material to be preserved.

In case the concentration of NaCl in a salt solution is found to be too low and if alcohol is detected in the stomach contents preserved with the same, it is necessary to determine the quantity of alcohol present in them and also to find out if urine, blood, liver and other organs contain any alcohol. In cases of alcohol poisoning, it is not in the stomach alone that the analyst will detect alcohol but in the other organs too. At the same time, he should inspect the condition of the lining membrane of the stomach which in alcohol poisoning is always found congested.

The ordinary 'methyiated spirit' containing pyridine bases and caoutchoucine (distillate of scrap rubber) which are used as lenaturants in this country is a highly undesirable preservative. The pyridine bases may be mistaken for alkaloids and thus interfere with the detection and identification of an alkaloidal poison.

The material for detection of gaseous poisons, e.g., blood for carbon monoxide, should not be mixed with any preservative, but it must reach the laboratory within a few hours and must be examined before decomposition sets in.

Importance of History and Clinical Indications in a Case of Poisoning

As poisons are numerous and as the chemical nature of most of the indigenous poisons is unknown, the medical or

the investigating police officer must as a rule supply all relevant informations regarding the suspected poison, the clinical signs and symptoms developed by the victim or the pathological findings recorded in post mortem examination, without which the toxicologist has to grope in the dark and to go on testing for all the poisons he knows of. This would naturally involve considerable waste of the poison which is usually too scanty even for a normal routine analysis, and thus the chances of missing it would be greatly increased, particularly if it is a rare or an indigenous poison. It may be noted that an accurate history of the case with correct clinical indications is rarely rendered available to the toxicologist in this country and more often than not the history supplied by the friends and relatives of the victim is misleading.

Before taking up the actual analysis of the viscera, the toxicologist must look for any congestion or corrosion that might have been produced by the poison in the stomach or intestines and he *should himself inspect the mucous coat of the stomach and the intestines instead of depending entirely on the post mortem report.*

General Considerations for Chemical Analysis

All chemicals required for analysis should be chemically pure and carefully stored in a special cupboard. The cleaning of glass-ware should be supervised by a responsible officer. No poisons should be kept in the room in which chemical analysis is conducted and no menials should be allowed to handle any of the reagents and the materials for examination. The beakers, basins, flasks, etc., employed in the poison extraction chamber, which is really a large fume chamber fitted with electricity, gas and water, should be properly marked and numbered to indicate the different stages of the experiment and also the nature of the materials which are being analysed there. As a case of fatal poisoning may comprise varieties of articles such as viscera, stomach-wash, vomited matter, urine, faeces, cups, plates or bottles, and suspected medicine or food, proper labelling or numbering of each of these articles is essential. *The analyst should never depend on his memory for identifying the beakers, flasks or*

basins used in keeping the solutions or extracts connected with any of the aforesaid exhibits so that no error might be introduced into the investigation. Blank tests should be carried out frequently or with each new set of chemicals and utensils to guard against contamination with any extraneous poison. To avoid accident and waste of materials, *shaking with solvents for the extraction of poisons should always be done in a mechanical shaker and not by an assistant.*

The material for examination should be divided into three portions: (1) *One portion to be examined for volatile and mineral poisons,* (2) *another portion for non-volatile organic poisons,* and (3) *the third portion to be held in reserve for certain inorganic poisons and for repeating, if necessary, any of the above items, or for carrying out any special experiment for the detection of any unknown indigenous poison, as for instance, the feeding of experimental animals with extracts prepared with different solvents.*

Routine Procedures for Chemical Analysis

(1) Gaseous Poisons.

The material for detection of gaseous poisons should be taken up first. As no preservative is added, it is quickly decomposed and thus rendered unfit for examination. *The usual poison in this country is CARBON MONOXIDE and the material is BLOOD.*

(2) Volatile Poisons.

(a) The material for volatile poisons, such as alcohol, phenol, aldehydes, chloroform, hydrocyanic acid and cyanides, chloral hydrate, phosphorus, ammonia, etc., should be taken up next. As no definite opinion can be given in most cases of poisoning without a quantitative determination of the poison detected, *it is necessary that the total quantity of each of the materials received and of the portions taken for analysis should be weighed or measured accurately and recorded.* The viscera (properly minced), stomach contents, vomit or other materials to be examined are not to be mixed together but should be examined separately. They should be brought to the consistency of a thin gruel by adding 3—5 times of distilled water and acidified with tartaric acid and submitted to steam distillation. The condenser and the receiving flask

should be well-cooled, especially during the hot season, with ice, the outlet of the condenser being dipped in a little water or NaOH solution or any other reagent as necessary. A few cubic centimetres of liquid paraffin and a few small pieces of pumice stone (ignited) may be taken in the flask to prevent frothing and bumping. It is better to collect the distillate in 4 or 5 fractions of which the first one should not exceed 20 c.c., and the remaining fractions should be of about 50 c.c. each. The flask containing the material should preferably be heated on the water bath. If phosphorus is suspected, the distillation should be carried out in a dark room and a black screen placed between the burners and the condenser (see p. 550) so that the phosphorescence may be seen quite clearly.

(b) The distillate from the acid mixture may contain alcohols, paraldehyde and other aldehydes, ethers, acetone, carbolic acid and other monohydric phenols (polyhydric phenols are not volatile in steam), carbon disulphide, thymol, camphor, turpentine, essential oils, caoutchoucine (from denatured spirit) and other volatile hydrocarbons, chloral, chloroform, nitroglycerine, benzene, benzoic acid, salicylic acid, hydrocyanic acid, hydrochloric acid and other volatile acids, phosphorus and also a few other volatile substances.

(c) After the completion of the acid distillation, the flask is allowed to cool and its contents *are rendered distinctly alkaline by adding NaOH solution or magnesia*. The alkaline mixture is then distilled again in the same way as before and the distillate collected in two fractions—the first fraction of about 20 c.c. and the second of about 50 c.c. The distillate from the alkaline mixture may contain aniline, pyridine, nicotine, coniine, ammonia and other volatile bases.

(3) *Inorganic Poisons.*

(a) The residual material in the distilling flask may now be submitted to tests for inorganic poisons. A complete analysis for all possible poisons is not necessary *because it is only rarely that more than one poison is administered to cause death*. The best procedure is, therefore, to seek for the most common poisons which the toxicologist usually comes across in his field of work and a few preliminary qualitative tests

may first be employed for this purpose, and of *these the Reinsch test occupies the foremost place*. It is the most convenient and valuable test for arsenic, antimony, bismuth and mercury and to some extent for silver.

(b) Copper salts may also be detected at this stage by what is called the *iron wire test*. The material is diluted with water, acidified with HCl and thoroughly shaken. It is filtered and in the filtrate a piece of clean and polished iron wire is placed and kept there for some time or overnight if necessary. In the presence of a copper salt, the iron will become coated with red metallic copper. The test is crude no doubt, but is quite reliable, if positive.

(c) If from the history of the case, or from the appearance, colour or smell of the original material, any suspicion of the presence of any other inorganic poison is roused, it will be desirable to submit this residual material to specific tests for the particular poison suspected. The *following metals may be remembered in this connection: barium, zinc, bismuth, lead, chromium, manganese, etc.* If the specific tests fail to detect any of these poisons, and which is not unlikely in view of the fact that poisons are usually scanty and always mixed up with large quantities of organic matter, such as the viscera, food-stuffs, fæces, etc., it will be necessary to submit the material to special methods (see para e) for the destruction of the organic matter and thereby setting free the metals as their inorganic salts for identification. In case a complete and systematic analysis of mineral poisons is called for, *the oxidized material is most suitable for the separation of the different groups of metals and their subsequent identification*.

(d) Besides the metallic poisons described above, there are several other inorganic compounds which require special attention, and they should be looked for in the third portion of the original material held in reserve (see p. 418). In this category come such compounds as *chlorates, nitrates and nitrites, sulphides, halides and the mineral acids*. Some of them are best separated from the mass of organic matters by *dialysis*. The material (specially the stomach contents and the vomit) if it is too thick in consistency, is diluted with

distilled water and is placed in the inner vessel of the dialyzer which is suspended inside the outer vessel containing distilled water. A dialyzer may be easily prepared in the laboratory by knocking off the bottom of a beaker and then tying tightly a wet parchment at this end with a piece of strong twine so as to permit of no leakage. The crystalloids pass out of the materials under examination from the inner vessel into the distilled water outside which is tested for the poison looked for.

(e) The Destruction of the Organic Matter of viscera, stomach-contents, fæces, etc., referred to in para (c), should always be done to set free the metallic poisons completely if a quantitative estimation of the poison is required, or if the Reinsch test or any other qualitative test for a suspected metal becomes doubtful or negative. There are several methods recommended for this purpose, of which 'wet ashing' or acid digestion is the best. Formerly, the method of *Fresenius and Babo* (oxidation by KClO_3 and HCl , see p. 569) used to be considered most suitable, but on account of its lengthy procedure, its inability to oxidize completely all the organic matter, the partial loss of arsenic during the process of digestion and the formation of a large quantity of KCl which saturates the final solution, this method is not much advocated nowadays and is made use of only in special cases, as for example, for the determination of mercury. Instead, the *nitric-sulphuric acid digestion method* of *Ramberg* (modified) is now in vogue. This method is quite satisfactory for the detection of all the metals except mercury which volatilizes during the process of oxidation of the organic matter. 100-200 grams of the material are weighed into a large Kjeldahl flask and 20 to 40 c.c. of conc. HNO_3 are added to cover the material and the flask is gently heated on a small flame when the mass begins to liquefy. The heating is continued until the liquefaction of the material is complete and that must be done in the presence of copious brown fumes of nitric oxide in the flask. At this stage, about 20-30 c.c. of conc. H_2SO_4 are added and the flask is heated strongly over a wire gauze, and conc. HNO_3 is added in drops (by means of the apparatus shown on page 422) to the contents of the flask at the rate of about 10 drops per minute so that the 'atmosphere in the flask must

at no time be free from brown fumes'. Heating is continued until all organic matter is destroyed and the liquid becomes clear and colourless or straw coloured. To find out if the

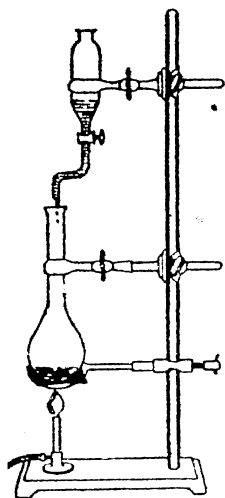
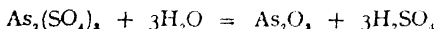


Fig. 13

oxidation is complete, the flask is heated without adding any HNO_3 . If there is any unburnt organic matter, the liquid begins to darken, and if the digestion is complete no darkening takes place and the white fumes of SO_3 are given off. In the former case, the addition of HNO_3 and heating are continued further till the organic matter is completely oxidized. Strong heating is continued for 15 minutes more to expel the nitric acid completely. Then, after cooling, 25 c.c. of saturated ammonium oxalate solution is added and the liquid is boiled until SO_3 fumes appear. This ensures complete removal of HNO_3 . It is then cooled, diluted with an equal volume of water and carefully trans-

ferred to a silica or pyrex beaker. The beaker is then heated on a hot plate or on a sand bath to expel the excess of H_2SO_4 . The solution is cooled and diluted with water in such a way that the strength of the acid in the mixture is in the neighbourhood of 10 per cent. At this stage a precipitate may be formed which contains the insoluble salts of lead, bismuth, tin, barium, strontium or silver. Arsenic, if present, will be in the soluble form as arsenic acid and an unstable sulphate $\text{As}_2(\text{SO}_4)_3$ which on dilution with water breaks down into the trioxide As_2O_3 :



The precipitate is filtered off and tested for the metals mentioned above. The filtrate will now contain all the other metals except mercury. For the detection of arsenic by Reinsch's test or for its estimation by Gutzeit's or Marsh's method, the arsenic acid present in the filtrate should be reduced to arsenious acid by treatment with a suitable reducing agent (see p. 425). The solution is now ready for systematic analysis for metals.

(f) For **Systematic Analysis for Metals** the usual procedure is first to reduce the acidity of the solution, if it is too strongly acid, by partially neutralizing with Na_2CO_3 , so that tin and antimony may be precipitated completely by H_2S . *In a strongly acid solution, Sb and Sn do not readily combine with H_2S .* A concentration of acid corresponding to a 0.25M solution is most satisfactory for the precipitation of these metals as sulphides. The following method is useful:

(i) The acid solution treated as above is heated on a water bath to a temperature of 60° — 70°C and then saturated with H_2S by passing the gas (arsenic free) for about an hour at this temperature. It is then removed from the water bath and *allowed to cool gradually, the passage of H_2S being continued until the solution is cold.* The flask is corked and set aside overnight. The precipitated sulphides settle down completely and are filtered off and then washed with water charged with H_2S . The residue on the filter consists of sulphides of As, Sb, Sn(part), Hg, Pb(part), Cu(part), Bi and cadmium, *while the filtrate contains chromium and zinc along with other metals with which we are not concerned.*

(ii) The sulphides upon the filter are thoroughly extracted with a hot mixture of ammonia and yellow ammonium sulphide. The filtrate is reheated and poured again over the precipitate on the filter, and this process is repeated several times. The final filtrate contains As, Sb, Sn and Cu and the residue on the filter paper contains Hg, Pb(part), Bi and Cd. The specific tests for these metals may now be applied to the filtrate as well as to the precipitate in the usual manner of qualitative analysis.

(iii) The filtrate from the H_2S precipitate containing chromium and zinc may be tested for the identification of these two metals.

(g) **The Reinsch Test.** It is best done in the following manner: About 20 c.c. of conc. HCl (pure for toxicological work) and 100 c.c. of water are taken in a porcelain basin in which a bright copper foil, about 3" by $\frac{1}{4}$ ", is placed with one of its ends being fixed on the edge of the basin in the form of a loop. It is boiled for about half an hour to see if the copper, basin, and

the acid are free from any of the metals the presence of which is sought for. In case there appears a stain on the copper foil, this blank experiment should be started again with fresh materials. If the blank is negative, the suspected substance is added and boiled for about an hour or more with occasional addition of water and acid to make up for the loss due to evaporation. If arsenic or any of the metals which are detected by this test is present, a stain will appear on the copper foil in a few minutes. In the case of arsenic, it will be a shining steel-grey stain which on further deposit of the metal becomes quite thick and black. The colour of the antimony stain is bluish black and that of bismuth is black and in the case of silver a dull or greyish white deposit of silver* is formed. In the presence of mercury, a bright silvery mirror is obtained. The copper foil is removed, carefully washed with distilled water and dried by pressing it gently between the folds of a filter paper. It is then cut into small strips and introduced in a small flat sublimation tube. The bottom of the tube is gently heated on a small flame; the arsenic, present on the foil as copper arsenide Cu_3As_2 ($2\text{AsCl}_3 + 6\text{Cu} = 3\text{CuCl}_2 + \text{Cu}_3\text{As}_2$), is oxidized and

crystals of arsenious oxide are deposited just above the heated portion of the tube. On examination under the low power of the microscope, beautiful octahedral and monoclinic crystals of As_2O_3 of varying sizes (see Fig. 44) will be observed. If the crystals are very small and scanty and if there is too much moisture present in the sublimate, due to incomplete drying of the copper foil before heating, the

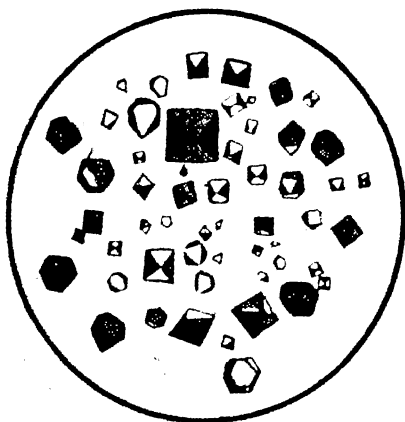


Fig. 44

crystals are likely to be missed under the microscope. This defect may be rectified by filling the tube with absolute alcohol

which absorbs the moisture and makes the sublimate clean for identification of the crystals, however small or ill-formed they may be.

Antimony, by this method, will sublime readily and yield characteristic needle-shaped crystals of Sb_2O_3 , either singly or in clusters (Fig. 62, p. 572). *Mercury* will give globules of metallic mercury. *Bismuth* does not sublime but may be oxidized, while the deposit of *silver* remains unchanged or turns black on oxidation.

There are certain limitations to this test which deserve careful attention. They are as follows: (i) The test does not work properly if oxidizing agents such as nitric acid, chlorates, MnO_2 , etc., are present as they tend to dissolve the copper foil during boiling. They must be removed before the test is applied, and the best way of removing them is to evaporate the material with excess of HCl to dryness, dissolve the residue in water and acidify it with HCl if necessary, but there is the possibility of some loss of arsenic during evaporation with excess of HCl . (ii) If pentavalent arsenic, i.e., arsenic acid, is present, the result is likely to be doubtful or negative. In such cases treatment with a suitable *reducing agent* such as sodium sulphite, potassium iodide, stannous chloride, ferrous sulphate, etc., will convert it into arsenious acid. It is, therefore, desirable to repeat the Reinsch test after treatment with a reducing agent in all cases where a negative result is obtained. (iii) Organic arsenical compounds do not also respond to this test but after the destruction of the organic matter (see p. 421) and conversion of the organic arsenical into an inorganic arsenic compound, the result becomes positive. (iv) Some organic sulphur compounds, on the other hand, produce a black stain of copper sulphide which may be mistaken for antimony or bismuth or even arsenic. (v) During the process of putrefaction of the tissues, arsenic may be converted into insoluble arsenic sulphides which may not respond to the test. Both (iv) and (v) may, however, be circumvented by previous oxidation of the material which removes the sulphur by oxidation. (vi) The concentration of HCl in the boiling mixture should not be too high or too low. It is desirable to maintain a concentration of

about 1 in 6. If the maximum limit is exceeded, there may be loss of arsenic as AsCl_3 by volatilization. If, however, there is no organic matrix in the material a concentration as low as 2 per cent of HCl may suffice for a positive reaction but it would certainly be a slow process, the optimum concentration being about 8 per cent in which no loss of arsenic takes place.

(4) *Non-volatile Organic Poisons*

This group comprises alkaloids, glucosides, barbiturates, salicylates, sulphonal group, acetanilide, sulphonamides, phenacetin, antipyrine, santonin, picric acid, picrotoxin, croton oil, certain animal poisons such as cantharides and snake venom, indigenous vegetable poisons of unknown composition, and a few other organic poisons. They are extracted in the following manner:

The extraction of these poisons depends on their solubility or otherwise in acid or alkaline water and in certain organic solvents which are immiscible with water. The method generally employed for extraction of the alkaloids and some glucosides is that of *Stas-Otto* or *Dragendorff*, which has been modified from time to time by different workers in this line. Both the methods are tedious and time-consuming and require much technical skill, particularly in cases where the poison is unstable and does not stand heat or when it undergoes hydrolytic decomposition during extraction. In the case of non-alkaloidal poisons, simple extraction with ether, chloroform or any other suitable solvent, without going through any of the long-drawn methods, may serve the purpose.

The main difficulty in all the methods for extraction of a poison from the viscera is to get rid of fats and pigments which interfere with its isolation in pure form and subsequent identification by confirmatory tests. The fact that most of the organic poisons, particularly the alkaloids, are generally found in very small quantities, stands in the way of repeating the processes for purification, as each repetition is followed by a certain amount of loss of the poison isolated giving a minus error. If not sufficiently purified, a positive error arises. This is the reason why the results of quantitative

determination of organic poisons especially the alkaloids are never dependable and the negative findings in many cases do not represent the actual state of affairs. The analyst "should, therefore, report his results as so much alkaloids actually recovered, and never state that the amount reported represents the exact quantity present in the tissues" (Webster).

The Stas-Otto Method of Extraction

The original method of Stas-Otto has been considerably modified and even now the toxicologists modify it according to their experience and to the nature of the material to be examined and also according to the nature of the alkaloid suspected to be present. The greatest difficulty is experienced in examining post mortem materials which contain too much organic matrix and too little poison. "There is 'analytic error' even in inorganic analysis, and when dealing with colloidal matter in masses, several tens of thousands times larger than the crystalloidal poisons to be recovered, the unavoidable 'analytic error' may exceed the ratio of the quantity of the poison, or leave less than what is needful for identification."

The following *modification* of the Stas-Otto method has been successfully employed for the routine examination of viscera and other materials:—

(a) The material is finely minced, preferably in a mincing machine, mixed with plenty of rectified spirit (about 2-3 times the weight of the material) in a flask and acidified with tartaric acid. The mixture is heated on the steam bath for 1—2 hours with thorough shaking at frequent intervals and the extraction is then allowed to proceed for about 24 hours with the steam off. It is then filtered through a fluted filter, the filtrate evaporated and the residue again extracted with acidulated alcohol in the same way, filtered and washed several times with hot rectified spirit. The combined filtrates are evaporated in a porcelain basin on the steam both to a syrupy consistency.

(b) To the syrupy residue about 100 cc. of rectified spirit is now added *very slowly with constant stirring* so that the insoluble matter may be granular and not gummy. If the alcohol is added *rapidly, or all at a time, the insoluble matter*

will be gummy causing much loss of alkaloids by enclosing them in the sticky mass. It is warmed with occasional stirring for about half an hour and filtered. This process is repeated once more and the combined alcoholic extracts are evaporated almost to dryness.

(c) The residue is now dissolved in about 50 c.c. of water acidulated with dilute sulphuric acid and filtered after about an hour. The poisons are thus dissolved out by the acid aqueous solution which is transferred to a separating funnel and extracted with a suitable solvent, such as ether, chloroform, etc., in portions of about 20 c.c. The solvent would take up the following from the acid solution: Colouring matters, toxic oils and resins, salicylic acid and its derivatives (aspirin, salol, etc.), barbiturates, sulphonal, acetanilide, phenacetin, traces of weak bases such as caffeine, colchicine, narcotine and alkaloids of ergot, certain glucosides such as thevetin, and any fat which has escaped initial treatments for purification.

(d) The acid aqueous solution is then rendered alkaline with a solution of sodium carbonate or ammonia which would liberate the free base from its salt. The alkaline solution is now extracted with chloroform in the same way as in the previous stage. It will take up all the alkaloids except morphine (only a trace being extracted) and those feebly basic substances which are partially extracted from the acid solution. The extraction is repeated 2 or 3 times more.

(e) If morphine is suspected, it may be extracted at this stage by amyl alcohol or chloroform-ether (3:1) mixture or chloroform-alcohol (9:1) mixture. Of these, amyl alcohol is the best but as it is prone to form annoying emulsions the chloroform-ether mixture is used by many. If morphine is likely to be the only poison, the chloroform extraction (stage d) described above may be omitted altogether.

The combined chloroform or amyl alcohol extracts are evaporated to dryness and the residue is now ready for further purification and confirmatory tests.

(f) The evaporated chloroform extract is purified by dissolving it in about 20 c.c. of water acidulated with sulphuric acid and filtering through a small filter. The

filtrate is extracted with chloroform, first in acid and then in alkaline medium, as was done during the initial stages of the extraction. These extracts are evaporated to dryness and submitted to specific tests for different poisons.

Modifications of the Stas-Otto Method under Special Circumstances

In carrying out the Stas-Otto extraction, one should use his discretion in modifying the different stages of the method according to the history or the medicolegal aspects of the case. In certain cases the number of stages may be reduced considerably to avoid loss from repeated purification. In cases where there is neither any history nor any definite post-mortem indication available, it may be necessary to proceed with the analysis systematically without cutting short any of its stages or modifying any of its details. It is important to remember that "every organic poison, whether alkaloid or otherwise, and if alkaloid, whether in the state of the free base or of a salt, is invariably more or less soluble in every menstruum employed in purifying the material. It is commonly assumed that in shaking out an acid solution with ether, chloroform, or other immiscible solvent all the alkaloid salt remains in the aqueous solution; but this is far from true, some of the alkaloid salt being taken up by the immiscible liquid used. In this way a serious loss of the poison may occur, and if the amount originally present is small there may be even a complete failure to detect it, the losses being so considerable" (Haines).

The following modifications of the technique are sometimes necessary under certain circumstances:

(i) If aconite, belladonna, datura or cocaine is suspected, the alcoholic extraction of the tissues is to be carried out at room temperature (not exceeding 40°C) and not on the steam bath (see para *a* on p. 427) and preferably with absolute alcohol instead of rectified spirit so as to prevent, as far as possible, the hydrolysis of the alkaloids, and the evaporation of the alcoholic extract should be done under reduced pressure at room temperature—a Cenco pump being very helpful in this operation.

(ii) If the material is preserved in saturated salt solution, the first extraction with alcohol should be continued for two days, but if it is preserved in rectified spirit as is usually the case, 24 hours' extraction as described under (a) is sufficient.

(iii) The stomach contents usually containing too much fluid matter should be extracted with excess of absolute alcohol, otherwise the rectified spirit becomes too much diluted, more so if they are preserved in NaCl solution. If, however, rectified spirit is used for the extraction instead of absolute alcohol, the process should be repeated 3 or 4 times or more. The reaction of the stomach contents being normally strongly acidic, the addition of tartaric acid in the first extraction may not be necessary.

(iv) The stomach washings are likely to contain some solid particles (e.g. *datura* seeds, kernels of *oleander*, gritty particles of dry opium, and many other substances of this nature) which should be carefully picked up, ground in a pestle and mortar, and submitted to the extraction process separately. In no circumstances should the solid residue in the specimen of the stomach-washing be mixed up with the supernatant fluid. Similarly, the first washing should not be mixed up with the others. As a large quantity of water is present in the stomach wash it should not be got rid of by boiling but should be expelled during the usual process of alcoholic extraction, that is to say, the material is mixed with about double the quantity of absolute alcohol, acidulated with tartaric acid, and then allowed to evaporate either on a steam bath or at room temperature according to the nature of the suspected poison.

(v) The filtration of the alcoholic extracts may be hastened by using a Buchner funnel and a suction pump. Ordinary funnel with cotton wool or cotton wool and sand may also be used for the same purpose.

(vi) There is always a danger of an emulsion being formed during the process of shaking of the aqueous solution with the solvent, but it may be easily avoided if the following technique is followed: *In the first extraction the mixture is shaken very gently and not more than half a dozen times,*

and then allowed to separate and the solvent is drained off. The next extraction is also done in the same way, shaking not exceeding a dozen times. The third extraction may be a little more vigorous, shaking being continued for about a minute or two, but if any indication of an emulsion becomes visible the shaking should be stopped at once and the solvent drained off. If the extraction is repeated in this way with increasing force in shaking, no emulsion will form even after prolonged and vigorous shaking during the last 2 or 3 extractions.

If, however, a heavy emulsion is formed at any stage due to inadvertence of the analyst, it is better to pour down the emulsion into a shallow dish and expel the solvent by evaporation on the steam bath or by aeration at room temperature and to *begin the agitation gently with a fresh quantity of the solvent, instead of wasting time by fruitless attempts to draw off the solvent.*

(vii) Picric acid, oxalic acid, or any such poison which is soluble in ether but insoluble in chloroform may be extracted from the acid aqueous solution *c* (p. 428) direct by ether. It may be noted that the ether extract may also contain salicylic and other acids, some barbiturates, some neutral principles, and the feebly basic alkaloids as obtained in the case of extraction by chloroform.

(viii) In suspected cases of poisoning by indigenous vegetable poisons, the extraction of the aqueous solution should be done first with chloroform in the usual way and then with ether, because there are certain glucosides which are soluble in ether but not in chloroform. Similarly, the alkaline aqueous solution *d* (p. 428) should also be extracted separately with chloroform as well as with ether.

CHAPTER XXXIII

Identification of Poisons. Gaseous Poisons.

Corrosives: Acids, Alkalies, Salts.

Identification of Poisons

The inorganic poisons offer no difficulty in their isolation and identification. It is with organic poisons that the analyst experiences a lot of difficulty both in their isolation and identification by chemical tests. The *sine qua non* of a successful chemical test is the purity of the reagents used as well as of the poison isolated. The reagents in pure condition are no doubt easily available but an organic poison, particularly an alkaloid isolated from the viscera and purified to the same degree of purity, is rarely obtainable.

Besides, due to bacterial decomposition, proteins of animal tissues undergo peculiar changes and form what are called *cadaveric alkaloids* or putrefactive bases or the so-called *ptomaines*, some of which respond to chemical as well as to physiological tests for vegetable or true alkaloids. It has been stated that these alkaloids are soluble in water but insoluble in chloroform or ether and as such they cannot be extracted by any of these solvents either from the acid or from the alkaline aqueous solution (Sidney Smith). They cannot, therefore, possibly introduce any fallacy into the investigation, although it is described in most text books that these bases are extractable by the usual solvents of alkaloids and are liable to be mistaken for poisonous alkaloids of vegetable origin.

It may, however, be noted that cadaveric alkaloids are rarely poisonous except perhaps two, *neurine* and *mydalcine*, which produce signs and symptoms resembling those produced by atropine, muscarine, aconitine, etc. In the Chemical Examiner's Laboratory in Calcutta, necropsy materials from nearly 1,000 cases are examined every year, but no such bases appear to have been isolated from the highly decomposed

bodies which are so common in this part of the country especially during the hot months of the year. We, therefore, agree with Sidney Smith that cadaveric alkaloids are not likely to interfere with our analysis and expression of definite opinions thereon.

The suspected poisonous materials isolated by any of the extraction methods described before should be tested chemically by more than one test and also biologically whenever it is feasible. *One should never depend on the single chemical test but must attempt to apply as many tests as the amount of the poison isolated would permit.*

In the following pages the chemical and other tests for the identification of inorganic and organic compounds obtained in medicolegal cases have been described. The tests which do not always give satisfactory results, have not been discussed here. The poisons have been taken up serially according to the classification given in the previous chapter.

GASEOUS POISONS

Carbon Monoxide

It is produced by the incomplete oxidation of coal, coke, charcoal and other fuels. It is present in ordinary coal gas varying from 4 to 15 per cent. Fatal poisoning from carbon monoxide is usually accidental and sometimes suicidal but rarely homicidal. Sleeping in small rooms with doors and windows closed and having burning charcoal to keep off cold, has been the cause of quite a large number of cases of fatal carbon monoxide poisoning in the slums of Calcutta and elsewhere. Suicide by coal gas or "gassing" is fairly common in the West. People who are accustomed to breathe the vitiated air of the house or to live in atmospheres rich in CO can resist the action of this toxic gas to a considerable extent and are not "gassed" as readily as those who are used to live in a more healthy atmosphere. The poisonous action of CO is due to its power of turning out the oxygen from the O_2 -hæmoglobin, thus depriving the tissues of the oxygen which is carried to them by the red blood corpuscles.

The material for examination in cases of CO poisoning is *the blood to which no preservative should be added.*

Tests for Carbon Monoxide

(i) *Colour Tests*.—The blood in CO poisoning is of red or pink colour. It retains this colour even if it is highly diluted while the dark coloured normal blood looks yellowish under the same condition. The comparison is best made by looking down through the columns of the diluted blood in Nessler's tubes placed on a white paper.

(ii) *Tannic Acid Test*.—Take 1 c.c. of the suspected blood, dilute with 4 c.c. of distilled water, add 3 c.c. of a 1 per cent solution of tannic acid and shake the mixture well and leave aside. A pink or carmine red flocculent precipitate appears which gradually settles at the bottom and retains its colour indefinitely. *If the tube is kept sealed the red colour persists for years.* In the case of normal blood, the precipitate when it is first formed is red but its colour changes gradually and in 1–2 hours it becomes brown or grey. After 24 hours the colour changes to dirty brown or grey.

(iii) *Katayama's Test*.—The suspected blood is diluted with distilled water (1:50). Take 10 c.c. of the diluted blood, add 4 drops of orange-red ammonium sulphide solution (prepared by adding 2 grams of sulphur to 100 c.c. of yellow ammonium sulphide) and 4–6 drops of 30 per cent acetic acid just to render the mixture faintly acidic. Mix well and filter off the precipitated sulphur. *The filtrate of CO-blood will retain its red colour while the normal blood becomes green or grey.*

(iv) *Spectroscopic Test*.—Take a drop or two of the suspected blood, dilute with distilled water (50 to 100 times), take some in a small test tube and examine with an ordinary hand direct-vision spectroscope. CO-blood, if saturated, shows two well-defined absorption bands (known as α and β bands) between D and E lines, slightly differing in position from those of oxyhæmoglobin (the α band being slightly nearer to the violet end). On the addition of a drop or two of a freshly prepared solution of ammonium sulphide, hydrazine, sodium hyposulphite $\text{Na}_2\text{S}_2\text{O}_4$, or Stoke's reagent (solution of ammoniacal ferrotartrate), the two bands would not coalesce to form one broad band of reduced hæmoglobin as happens in the case of oxyhæmoglobin of normal blood.

It is important to remember that in cases of CO poisoning where the blood contains less than 30 per cent of CO-hæmoglobin, the result is confusing. In such cases the addition of the *reducing agent reduces the oxyhæmoglobin portion of the blood leaving the CO-hæmoglobin portion intact and so both the spectra appear on the scene and make the resulting spectra indistinct and ill-defined.*

To carry out successfully the spectroscopic test for CO-blood, it is better to take a drop or two of normal blood and gradually dilute it to the required strength so that the normal oxyhæmoglobin bands (between D and E lines) are distinctly seen in the spectroscope. Reduce it with a drop of freshly prepared solution of a reducing agent when the two bands coalesce to form one broad band characteristic of reduced hæmoglobin. *With the same amounts of suspected blood and the reducing agent and with the same extent of dilution as was found most suitable for normal blood, the suspected blood would give a satisfactory result.*

Quantitative Determination of CO in Blood

This may be done both by chemical and spectroscopic methods. For the latter method, Hartridge's Reversion Spectroscope serves the purpose best, the detailed description of which will be found in the Analyst (Vol. 56, p. 561, 1931). This method is quick and accurate. The qualitative tannic acid test may be made quantitative by fully saturating a sample of normal blood with carbon monoxide and then adding this sample to normal blood in different proportions varying from 10 to 90 per cent. The mixed samples are precipitated with tannic acid solution as described above and thus form the standards with which the depth of colour of the sample of suspected blood (treated in the same way and with the same strength of tannic acid solution) is compared.

For purposes of calculation it is necessary to remember the following data: (1) A blood containing the normal amount of hæmoglobin will retain 20 volumes per cent of CO after complete saturation. (2) A blood containing less hæmoglobin will require correspondingly less amount of CO for saturation. (3) The amount of CO in blood is expressed in terms of percentage of saturation, e.g., if the CO content of a blood is 12 volumes per cent, its percentage of saturation is 20:100::12:X, or 60, provided its hæmoglobin content is normal.

The other gaseous poisons are H_2S , PH_3 , and AsH_3 , but as cases of poisoning by these gases have not been recorded so far in this country they have not been discussed here.

CORROSIVES

CORROSIVE ACIDS

1. Sulphuric Acid

In cases of suspected sulphuric acid poisoning it is essential, for the expression of a definite opinion, to detect free sulphuric acid in the material (stomach-contents, vomit, or tissues) or to determine the total quantity of sulphates so as to prove that such an amount could not be accounted for in any way other than the administration of sulphuric acid.

Free sulphuric acid is rarely found in stomach contents or tissues after death for following reasons: (1) It may be vomited out, (2) may be neutralized by alkalies given as antidotes, and (c) may combine chemically with the tissues with which it comes in contact. On account of the presence of sulphates normally present in tissues and food materials, and of the free HCl of the gastric juice, it is difficult to state definitely that free H_2SO_4 is present.

In "acid throwing" cases the skin, blackened or discoloured by the acid and the blisters produced thereby, may be carefully snipped off with a pair of scissors and submitted to the method of extraction described below. As the quantity of the material available for analysis is too small, necessary modifications have to be introduced.

Extraction of H_2SO_4 and Soluble Sulphates.—By the following method of extraction, free sulphuric acid as well as soluble sulphates may be isolated and quantitatively determined:

(a) Weigh out finely minced tissues, stomach contents or any other material and extract with distilled water on the steam bath for a few hours and then leave it overnight. Filter and wash the residue with water till the filtrate is sulphate free. Concentrate the filtrate to about 150 c.c. and transfer to a measuring flask, rinse and make up the volume to 150 c.c. with distilled water (A).

(b) Measure out 100 c.c. of the filtrate and evaporate to as near dryness as is possible on the steam bath. The residue consists of free H_2SO_4 , sulphates and other salts with some

organic matter. The free HCl of the stomach and any volatile organic acids originally present in the material are all volatilized at this stage. Sometimes phosphoric acid is produced by the action of sulphuric acid on phosphates of tissues and foodstuffs and it is retained along with the sulphuric acid in the residue. Treat the residue with a mixture (1:1) of cold alcohol and ether. Filter and wash the insoluble matter (consisting of sulphates and other salts) with alcohol-ether mixture till the filtrate is acid free. Evaporate the filtrate on the steam bath and expel alcohol and ether completely, the residue being only a syrupy substance consisting of free H_2SO_4 and traces of ethyl hydrogen sulphate and H_3PO_4 . Add about 30 c.c. of water and heat the solution to boiling for hydrolytic decomposition of ethyl hydrogen sulphate into H_2SO_4 and alcohol, the latter being expelled by boiling. This acid solution (B) is now fit for detection of free H_2SO_4 and determination of its quantity. Divide it into two equal portions, one portion for qualitative tests for free H_2SO_4 and the other for its volumetric or gravimetric determination.

(c) Take the remaining portion (50 c.c.) of the filtrate (C) from the measuring flask (A) for determination of the total soluble sulphates (free and combined H_2SO_4) present in the material. By deducting the amount of free H_2SO_4 , i.e., (B) from the result of (C), the amount of sulphates would be obtained.

Tests for Free Mineral Acids

(i) Add a few drops of an aqueous solution of methyl violet (0.01 per cent) to 2-3 c.c. of the acid solution, a greenish blue colour is produced. Organic acids do not produce any change in colour.

(ii) Test the solution with congo-red paper, the red colour changes to deep blue. Organic acids produce a violet colour.

(iii) Add a few drops of cochineal solution, the red colour changes to yellow.

Tests for Sulphuric Acid

(i) Add a few drops of BaCl_2 or $\text{Ba}(\text{NO}_3)_2$ solution, a white precipitate of BaSO_4 insoluble in HCl or HNO_3 is formed. Free H_2SO_4 and all soluble sulphates give BaSO_4 .

(ii) Take some acid solution in a test tube fitted with a bent tube (bent twice at right angles) and concentrate it to a syrup by boiling. Add a small piece of a copper turning and boil again, SO_2 is liberated which is passed into another test tube containing

- (a) some stannous chloride solution; on warming it gives a yellow precipitate, or
- (b) some iodic acid solution with 2-3 c.c. of chloroform; SO_2 reduces iodic acid and liberates iodine which imparts a violet colour to chloroform on shaking, or
- (c) some weak solution of KMnO_4 (about 0.1 per cent or less); the pink colour of permanganate is discharged by SO_2 .

Quantitative Determination of H_2SO_4 and Soluble Sulphates

Measure out and transfer the solutions (B) and (C) to two beakers, acidulate with HNO_3 , heat to boiling and add $\text{Ba}(\text{NO}_3)_2$ solution drop by drop till the precipitation of SO_4 as BaSO_4 is completed in both the beakers. Allow to cool. Filter and wash till the filtrates are free from acid. Dry and ignite the residues. As there are some organic matters in the solution, particularly in (C) there would be slight reduction of sulphates to sulphides which should be reconverted to sulphates in the usual way. The weight of BaSO_4 in solution (B) represents the amount of free H_2SO_4 , and the weight of BaSO_4 in solution (C) represents the total amount of free and combined H_2SO_4 . Multiply the weight of BaSO_4 by 0.4201, the result will give the weight of H_2SO_4 in the quantity of the solution examined, or by 0.3420 and the result will be the weight of H_2SO_4 or sulphates calculated as SO_4 .

The amount of free H_2SO_4 present in the solution (B) can also be determined by titration with a standard alkali but as H_3PO_4 and in some cases traces of lactic acid are likely to be present, the gravimetric method is to be preferred.

N.B. The normal sulphate contents (calculated as SO_4) of liver, kidney, spleen and brain are, according to Witthaus, 5.2, 11.5, 0.0, and 18.7 milligrams respectively per 100 grams of the tissue.

2. Nitric Acid

Free nitric acid is, as in the case of H_2SO_4 poisoning, not often found in the stomach. Much of it combines with the proteins of the tissues and food materials to form xanthoproteic acid and nitro compounds.

Extraction of Free Nitric Acid.—*Free nitric acid* may, however, be detected by test papers in the same way as described under H_2SO_4 . If a free acid is indicated, it should be extracted and confirmed by the following method (Baumert): The tissues, stomach contents, etc., are extracted with distilled water on the steam bath for some time and filtered. Neutralize the filtrate with slaked lime or calcium carbonate and evaporate to dryness. *Extract with cold 95 per cent alcohol in which calcium and sodium nitrates are soluble.* Filter the alcoholic extract and again evaporate to dryness. Dissolve the residue in distilled water. Filter the aqueous solution and evaporate to dryness. Dissolve the residue in a mixture of *alcohol and ether (1:1) in which only calcium nitrate is soluble*, transfer it to a well corked flask and keep it overnight. Filter, wash with alcohol-ether mixture and evaporate the filtrate to dryness. Dissolve the residue, $\text{Ca}(\text{NO}_3)_2$, in a small amount of water and test for nitrates.

Tests for HNO_3 and Nitrates

(i) *Brucine Test.*—Take a drop of the solution in a porcelain dish, add a drop of conc. H_2SO_4 (chemically pure) and a small crystal of brucine—a blood-red colour is produced if nitrate is present.

(ii) *Ferrous Sulphate Test.*—Take a few drops of the solution in a porcelain dish, add a drop or two of conc. H_2SO_4 and a crystal of ferrous sulphate when a dark brown colour develops round the crystal due to formation of $\text{FeSO}_4 \cdot \text{NO}$.

This test may be done in the usual way in a test tube if a sufficient amount of the nitrate is available, when a dark brown ring develops at the junction of the concentrated sulphuric acid and nitrate-ferrous sulphate mixture.

(iii) *Copper Test.*—Take the solution in a test tube, place a small piece of copper turning and add a drop or two of pure conc. H_2SO_4 to liberate HNO_3 from the nitrate and boil. HNO_3 becomes concentrated and attacks copper to form reddish brown fumes of NO_2 . If the amount of

nitric acid is too small in quantity, the brown fumes will not be visible but may be easily detected by starch-iodide paper which turns blue.

(iv) *Diphenylamine Test*.—Add a few drops of the diphenylamine reagent to the nitrate solution and layer this mixture over some pure conc. H_2SO_4 in a test tube when a blue colour appears at the junction of the acid and reagent layers. This reagent is prepared by dissolving 1 gm. of diphenylamine in 5 c.c. of dilute H_2SO_4 (10 per cent) and 100 c.c. of water.

This test is very delicate but is *not specific for nitrates*. It is also given by other oxidizing substances, e.g., chlorates, nitrites, permanganates, chromates, peroxides, etc.

If there is much excess of nitric acid, it may easily be detected by filtering and washing out the materials with distilled water and submitting the filtrate to the specific tests described above, or it may be isolated in purer condition by ordinary distillation. If there is no indication of any free acid, the material should be tested for nitrates and, if positive, the determination of the amount of nitrates would be necessary to prove that such an amount could only be due to administration of nitric acid or nitrates.

Quantitative Determination of Nitrates and Nitric Acid

Dumreicher's Method.—Carefully extract a weighed amount of the material (finely minced tissues, stomach contents, vomit, etc.) with distilled water for a few hours on the steam bath. Filter and thoroughly wash the residue with water. Transfer the filtrate to a flask and add excess of freshly prepared stannous chloride solution (prepared by dissolving 16 g. pure granulated tin in 60 g. of 40 per cent pure HCl) and boil the mixture for an hour. During this process nitric acid and nitrates are reduced to ammonia which combines with HCl to form NH_4Cl . Transfer the mixture to a porcelain basin, and evaporate on the steam bath to dryness, dissolve the residue in distilled water, filter and wash. Transfer the filtrate to a flask and render it alkaline with strong (40 per cent) solution of caustic soda and distil. Collect the distillate in a conical flask containing a known amount of $\text{N}/10 \text{ H}_2\text{SO}_4$ with a few drops of an indicator. Titrate it with $\text{N}/10 \text{ NaOH}$. Multiply the amount of ammonia determined by this titration by 3.7, and the result will represent the quantity of HNO_3 present in the weighed amount of the material taken.

The presence of large quantities of organic matter from tissues or food materials may prevent successful extraction of nitrates or their reduction to ammonia for quantitative determination. In such condi-

tions the use of a dialyzer for separation of nitrates in pure form from the mass of organic matters may be helpful.

The skin stained yellow with HNO_3 or blisters from the skin in 'acid throwing' cases may also be examined for detection of nitrates by modifying the processes and tests described in the preceding paragraphs.

3. Hydrochloric Acid

Since free hydrochloric acid is normally present in the stomach to the extent of about 0.4 per cent, and since chlorides are always present in tissues and foods and also taken sometimes in excess by some people as a condiment a quantitative analysis is imperative, much more than in the case of sulphuric or nitric acid poisoning, before anything significant is assumed.

If the reaction of the material is acid and if the acidity is due to free HCl as indicated by its specific tests described below, quantitative determination of both free HCl and soluble chlorides has to be carried out. If the reaction is neutral or alkaline (due to alkalies administered as antidote), the question of free HCl does not arise and the amount of total chlorides requires to be determined.

Extraction.—The liquid materials, e.g., stomach wash, may be tested directly after repeated filtrations but if the result is not very decisive, distillation is necessary. The solid material should be mixed with water and distilled as described below:

Distil a portion of the material (tissues properly minced, stomach contents, vomit, stomach-wash, etc.) and collect the distillate in two fractions—the first fraction consists mainly of water and the last fraction consists of water and HCl. The distillation in such cases is to be continued till the contents of the flask are almost dry so that the free HCl may be distilled completely, the last few c.c.'s of the distillate being usually the pure acid.

It may be noted that HCl does not begin to distil until its concentration is about 10 per cent. If, for instance, 100 c.c. of a solution containing 1 per cent HCl is distilled, the first 90 c.c. of the distillate contains, if at all, only traces of the acid while the last fraction would contain

the whole of the acid which may be submitted to the following tests :

Tests for free HCl and Chlorides

(i) *Congo-red paper test* or any other suitable test for free mineral acids as described under sulphuric acid.

(ii) *Gunzberg's Test*.—Take a few drops of Gunzberg's reagent in a porcelain dish, evaporate to dryness over a small flame and cool. Add with a glass rod the material to be tested to the dried reagent. Warm gently—a purplish red colour develops in the presence of free HCl.

N.B. H_2SO_4 also gives this test to a certain extent.

Gunzberg's reagent is prepared by dissolving 2 g. of phloroglucinol and one gram of vanillin in 100 c.c. of 95 per cent alcohol.

(iii) *Silver Nitrate Test*.—Add a few drops of AgNO_3 solution—a curdy white precipitate is obtained which is soluble in ammonia or KCN and insoluble in strong HNO_3 even on boiling.

N.B. HCN also gives a white precipitate under the same conditions but it is soluble in HNO_3 on heating.

(iv) *Chlorine Test*.—Add a pinch of powdered MnO_2 and boil. When the solution is sufficiently concentrated after the excess of water is driven off by boiling, HCl is acted upon by MnO_2 and free chlorine is liberated which is detected by its characteristic smell and colour. But if it is evolved in a very minute quantity, its presence can be detected by a delicate test such as starch-iodide paper which turns blue.

Quantitative Determination of HCl and Chlorides

Weigh out the material and extract with warm water for an hour or two with frequent shaking. Filter into a measuring flask and wash the residue till the filtrate is free from chlorides as indicated by a drop of AgNO_3 solution. Make up the required volume by adding distilled water.

(a) Take an aliquot part (about $\frac{1}{3}$ of the total bulk) and neutralize (if it is acid in reaction due to presence of free HCl) with sodium carbonate to fix up the free HCl (volatile) as sodium chloride. Evaporate it to dryness in a platinum or porcelain capsule, incinerate to complete carbonization of organic matters which are

always present in such filtrates, breaking up with glass rod the lumps of carbonized mass formed during incineration. Extract the carbonized residue with small portions of distilled water till the filtrate is chloride free. Acidify with HNO_3 and add AgNO_3 solution in slight excess which converts all soluble chlorides into insoluble AgCl . Filter and wash the residue (AgCl) till it is acid free. Dry and weigh according to the usual methods of gravimetric analysis. The weight of dried AgCl thus obtained represents the weight of free HCl and chlorides taken together.

(b) Take another portion of the filtrate (exactly the same volume as taken in (a) and evaporate to dryness without previous neutralization. This will drive off free HCl and also other volatile acids if present. Incinerate and proceed in the same way as in the previous experiment. The weight of AgCl now obtained represents only the soluble chlorides. If this figure is deducted from that of (a), the difference would represent the amount of free HCl .

This method of determination of free HCl and chlorides is open to an objection that a considerable quantity of HCl is retained by tissues and other organic matters in the form of acid-albumin and as such the stage (a) represents free HCl , chlorides and acid-albumin, and the stage (b) represents only the chlorides and that the difference between (a) and (b) does not represent the free HCl only but free HCl plus acid-albumin. To obviate this difficulty, the method has been modified (Winter—Hayem) by introducing another stage (c) to indicate the amount of acid-albumin and chlorides taken together, and is as follows:

(c) Take the third portion of the filtrate [same volume as taken for (a) or (b)] and evaporate to dryness on the steam bath and leave it there for an hour or more (free HCl is expelled leaving acid-albumin and chlorides in the residue). Add excess of sodium carbonate solution, and thoroughly mix with the residue and evaporate to dryness. Incinerate to complete carbonization and then proceed as in (a).

The experiments (a), (b) and (c) would give the following results: Free HCl = (a) - (c), chlorides = (b), and acid-albumin = (c) - (b). It may, however be stated that ordinarily this extra precaution for accuracy is necessary in biochemical but not in chemicolegal investigations.

The following factors may be noted for calculating the results: The weight of dried AgCl , if multiplied by the factor 0.2543 gives the weight of anhydrous HCl , by the factor 0.8 gives the weight of concentrated HCl (B.P.) which contains 31.9 per cent of anhydrous HCl , and by the factor 0.4075, gives the weight of soluble chlorides calculated as NaCl .

4. Oxalic Acid

The detection of oxalic acid or oxalates in stomach contents, urine, tissues, etc., is only a presumptive evidence

of oxalic acid poisoning as oxalates are widely distributed in nature. All plants, particularly the vegetables which we take, contain appreciable amounts of calcium and potassium oxalates. As insoluble calcium oxalate is soluble in dilute HCl, the presence of free oxalic acid in the stomach may to a certain extent be also due to the action of free HCl of the gastric juice on calcium oxalate of foodstuffs. In this peculiar circumstance, quantitative determination of total oxalates is absolutely necessary for any opinion as to the cause of presence of oxalic acid in the material under examination. *It may, however, be noted that soluble salts of oxalic acid are also poisonous.*

In a case of poisoning, the oxalic acid may be present as (1) free oxalic acid, (2) as soluble oxalates, and (3) as insoluble oxalates formed as a result of the administration of antidotes. It may be found in the stomach contents, stomach wash and vomit mostly as free acid, in the liver, kidneys and other tissues mostly as soluble oxalates, and in the urine mostly as insoluble oxalates.

Extraction of Oxalic Acid and Oxalates.—The acid in its three different forms may be isolated by the following method: Finely mince the solid material, add 3 to 4 volumes of distilled water and digest on a water bath for an hour with frequent shaking. Filter. The filtrate (A) will contain the free acid and its soluble salts, and the residue (B) will contain the insoluble oxalates. Evaporate the filtrate (A) to dryness on the steam bath, cool, and add excess of absolute alcohol and digest for about an hour for complete solution of the free acid. Filter; this filtrate (alcoholic) will contain the free acid, and the residue (alcohol insoluble) will contain the soluble oxalates. Evaporate the filtrate to dryness and dissolve it in a small quantity of distilled water; this aqueous solution now contains the *free acid*.

The alcohol-insoluble residue containing the soluble oxalates is digested with alcohol acidified with HCl to liberate oxalic acid. Filter and evaporate the filtrate to dryness. Dissolve it in distilled water; the aqueous solution now contains the *soluble oxalates* in the form of free oxalic acid.

Take the water-insoluble residue (B) in a beaker, add 3 to 4 volumes of water, add a few c.c. of sodium carbonate solution to make it distinctly alkaline and boil for about 2 hours to convert the insoluble oxalates to their soluble form. Cool, filter, and evaporate the filtrate to dryness. Add 3-4 volumes of alcohol, acidify with HCl and digest for about an hour. Filter, evaporate the filtrate to dryness and dissolve the residue in distilled water; this aqueous solution contains the insoluble oxalates which have been converted into the soluble form and then into oxalic acid.

Tests for Oxalic Acid and Oxalates

(i) Add lime-water, calcium acetate, or a saturated calcium sulphate solution to oxalic acid or soluble oxalates—a white precipitate of calcium oxalate is formed. The precipitate is insoluble in acetic acid and ammonia but soluble in dilute HCl and HNO_3 .

(ii) Neutralize oxalic acid with ammonia, add CaCl_2 solution—a white precipitate of calcium oxalate is formed.

(iii) Add silver nitrate solution to a neutral solution of oxalates—a white precipitate of silver oxalate soluble in ammonia is formed.

(iv) Add a few drops of a dilute solution of KMnO_4 to a solution of oxalic acid or oxalates, acidify with dilute H_2SO_4 and heat—the colour of the permanganate is discharged.

(v) Take about 5 c.c. of the solution of oxalic acid, mix with 1 c.c. of dilute H_2SO_4 (1 : 2) and add 2 drops of 10 per cent CuSO_4 solution. Put into the mixture about 1 g. of granulated zinc (to form zinc-copper couple). After 3 minutes add 2 c.c. of conc. H_2SO_4 to the mixture and 0.1 c.c. of a 2 per cent aqueous solution of resorcinol, a pale blue colour develops which deepens on warming (Analyst, 1937).

Quantitative Determination of Oxalic Acid

The determination of the amounts of oxalic acid present in three forms can also be made by the same methods described before, taking of course the necessary precautions against loss at different stages of the experiments. But for practical purposes and for reasons discussed above the determination of the amount of total oxalic acid (free and combined) is usually considered sufficient.

The following methods for the determination of oxalic acid are useful:

(i) **For viscera**—Weigh out about 100 grams of the finely minced viscera, stomach contents or vomit, and add 3-4 volumes of alcohol and acidify with HCl. Digest in the cold for about 2 hours. Filter through fluted filter paper and wash the residue with alcohol. Add about 20 c.c. of water to the filtrate (to prevent the formation of ethyl oxalate during evaporation) and evaporate on the steam bath to expel the alcohol completely. Filter and transfer the filtrate (which is about 20 c.c. in all) to a separating funnel and extract 3-4 times with 50 c.c. portions or more of ether. Combine the ether extracts and filter through a dry filter paper. Evaporate or distil. Dissolve the residue in 2-3 c.c. of distilled water. Render it distinctly alkaline with ammonia and then add saturated CaSO_4 solution or 10 per cent CaCl_2 solution till the precipitation of calcium oxalate is completed. Acidify with acetic acid and leave the solution with its precipitate for 24 hours in a covered beaker. Filter. Wash the residue till it is acid free, dry and ignite in the usual manner to convert calcium oxalate into CaO and weigh as CaO . The weight of CaO multiplied by 2.25 gives the weight of crystallized oxalic acid ($\text{C}_2\text{H}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) present in the weighed amount of the material. If multiplied by the factor 1.61, the weight will represent the amount of anhydrous oxalic acid.

(ii) **For the urine or stomach-wash**—*Salkowski's method* is useful: Take 500 c.c. of the urine and concentrate to about $1/4$ or $1/3$ of its bulk. Cool, add 20 c.c. of HCl and extract five times with an equal volume of ether-alcohol (9:1) mixture. Filter the ether-alcohol extracts through a dry filter and evaporate or distil the filtrate to expel ether (leaving out the alcohol portion). Add 10-15 c.c. of water to the alcoholic residue and transfer it to a beaker and concentrate on the steam bath until the separation of a resinous material, water being added from time to time during this process of concentration. Cool and dilute the liquid with about 10 c.c. of water and filter with 2 or more washings, the total volume not exceeding 20 c.c. To the filtrate rendered faintly alkaline with ammonia, add drop by drop 1-2 c.c. of a 10 per cent solution of calcium chloride till the precipitation of calcium oxalate is complete. Acidify with acetic acid and allow to stand for 24 hours. Then proceed as in the case of viscera.

The residue of calcium oxalate in the filter, if found quite clean and white, may be estimated volumetrically by dissolving it in hot dilute H_2SO_4 and titrating (on a water bath at a temperature of about 60°C) with $\text{N}/10$ KMnO_4 solution. 1 c.c. of $\text{N}/10$ KMnO_4 solution = 0.0063 gram of crystallized oxalic acid.

N.B. The acid ether extract by the Stas-Otto process already described (see pp. 428, 431) contains free oxalic acid. This extract may therefore be tested for oxalic acid. If poisoning by oxalic acid is suspected, the material should be examined directly for oxalic acid instead of submitting it to the Stas-Otto method of extraction.

5. Carbolic Acid, Phenol

Phenol may be found in the stomach contents mostly as free phenol and in the tissues mostly in combination with sulphuric acid, glycuronic acid, etc., which are formed as metabolic products, and partly as complex organic compounds produced by the oxidation of phenol and subsequent combination with acids. The detection of phenol both in the free state and in combination with acids is of medicolegal importance. In cases of acute carbolic acid poisoning, free carbolic acid may not always be present on account of administration of antidotes although it is indicated by its smell and signs of corrosion.

Extraction of Free and Combined Carbolic Acid

—Macerate the material with 5 per cent sulphuric acid and distil in steam. The distillate would contain free phenol which was originally present as such and also obtained by decomposition of combined products. Apply the following tests to the distillate for detection of phenol. If phenol is present in excess, it may be found as oily drops in the distillate.

Preliminary Tests

(i) *Ferric Chloride Test*.—Add drop by drop a very dilute solution of FeCl_3 to about 2 c.c. of the distillate—a violet or bluish violet colour is produced. The colour is discharged on adding alcohol (distinction from salicylic acid).

N.B. This property accounts for the fact why alcohol is not recommended as a preservative for post mortem materials in cases of carbolic acid poisoning.

The violet colour changes to yellow on adding a dilute mineral acid. *The FeCl_3 test becomes therefore negative in the presence of mineral acids and alcohol.*

Cresols, particularly ortho-cresol, and other phenols and also salicylic acid give this test, the shades of colour—violet to bluish violet, being different in each case. Antipyrine, pyramidon and similar non-phenolic compounds also give this test.

(ii) *Bromine-water Test*.—Add bromine-water drop by drop—a white precipitate which redissolves quickly appears on adding excess of the reagent. It is a crystalline precipitate of tribromophenol $C_6H_2Br_3OH$ (m.p. $93-94^\circ$), readily soluble in alcohol, ether, and alkali but insoluble in acids.

If a large excess of bromine-water is added as indicated by the yellow colour of the mixture, a precipitate of tribromophenyl hypobromite $C_6H_2Br_3OBr$ is formed which melts at $132-134^\circ$ with evolution of bromine.

Other phenols, cresols, salicylic acid, etc., give similar precipitates which, however, differ in their crystalline forms and melting points.

(iii) *Millon's Test*.—Add slight excess of Millon's reagent (about half the volume of the distillate to be tested) and heat for a few seconds—a red colour develops. If the amount of phenol in the distillate is not too small, the red colour may appear even in the cold.

All monohydric phenols and their derivatives, aniline and certain proteins also give this test.

(iv) *Liebermann's Test*.—See phenol (page 302).

(v) *Hypochlorite Test*.—Add drop by drop a slight excess of dilute ammonia (about $\frac{1}{4}$ th the volume) and 2—4 drops of a freshly prepared solution of bleaching powder and warm—a blue, bluish-green or green colour is developed according to the concentration of the phenol present in the distillate. Other phenols particularly the cresols, give positive reaction with this test.

Confirmatory Tests

(i) *Ware's Nitrite Test*.—Dissolve 1 drop (0.05 gm.) of the phenol in 5—10 c.c. of conc. HCl in a mortar, add a minute crystal of $NaNO_2$ (size of a pin's head) and stir—at once a yellow colour appears. Transfer the mixture to a test tube and heat slowly nearly to boiling—the yellow colour gradually changes to yellowish brown, brown, reddish brown and red. Cool, dilute with water, and pour drop by drop into excess of 10 per cent NH_4OH solution—a bluish green or deep blue colour appears.

Carbolic acid and cresol B.P. are the only phenols which give this test. The other phenols produce colourations which are quite different from those stated above. A positive nitrite test, therefore, indicates the presence of either carbolic acid or cresol B.P. The next two tests distinguish one from the other and thus confirm the presence of either.

(ii) *Ware's Nitrite-Nitrate Test.*—Dissolve 1 drop of the phenol in 10 c.c. of conc. HCl in a mortar, add 0.5 gm. of the nitrite-nitrate mixture (NaNO_2 1 part, NaNO_3 or KNO_3 1 part and anhydrous Na_2SO_4 2 parts), dissolve it and allow the mixture to stand for 2—5 minutes. In the presence of carbolic acid, a rich crimson or blood-red colour appears. (a) Pour 1 c.c. of the blood red mixture, drop by drop, into some 10 per cent NH_4OH solution—a deep emerald green colour is produced. (b) Add 1 or 2 drops of formalin (38—40 per cent formaldehyde) to about 2 c.c. of the blood-red mixture—the colour changes to beautiful purple. Pour the purple mixture, drop by drop, into some 10 per cent solution of NH_4OH , a greenish blue or deep blue colour is produced.

In the presence of cresol (B.P.) no blood red colour is produced, only brown or reddish brown colour appears. Addition of formalin does not change it to purple and pouring into 10 per cent ammonia changes it to yellowish brown. No crimson, emerald green, or blue colour is produced at any of its stages. This test, therefore, *distinguishes carbolic acid from cresol.*

In testing an extract from viscera, experiments with pure phenol or cresol should be carried out side by side for comparison.

(iii) *Benzaldehyde Test.*—Add double the amount of conc. H_2SO_4 to a small quantity of the distillate and 1—2 drops of benzaldehyde—a yellow or yellowish brown colour is produced. Heat to boiling—the colour changes to dark red and a red resinous substance separates out if the solution is not very dilute. Cool and add about 10 c.c. of water and render the mixture alkaline with NaOH—a beautiful violet-blue colour develops.

This test is almost specific for carbolic acid, the only other phenol that gives a positive reaction being pure ortho-cresol. The B.P. cresol does not give this test.

Quantitative Determination

Bromate-Bromide Method.—Weigh the material, mix with about 100 c.c. of water and add dilute H_2SO_4 so that the acidity of the mixture is about 5 per cent, and steam distil. The distillation should be continued till the last drop of the distillate gives no turbidity with a drop of bromine water. Measure the distillate and take an aliquot portion (25 c.c.) in a glass-stoppered flask of about 500 c.c. capacity. Add 30 c.c. of N/10 bromine solution (prepared by dissolving 2.784 grams of $KBrO_3$ and 10 grams of KBr in a litre of water) and 5 c.c. conc. HCl . Put in the stopper and shake frequently for about half an hour. The mixture becomes gradually opalescent and subsequently a precipitate is formed and the colour of the mixture changes to yellow. Open the stopper after 15 minutes, add 5 c.c. of 20 per cent KI solution, shake for a few minutes and titrate the free-iodine with N/10 sodium thiosulphate, using starch solution as an indicator. As each c.c. of N/10 thiosulphate is equivalent to 1 c.c. of N/10 iodine or N/10 bromine solution subtract the number of c.c. of N/10 sodium thiosulphate used in the titration from the number of c.c. of N/10 bromine originally added. Multiply the difference by the factor 0.001568 which would give the amount of phenol in 25 c.c. of the distillate taken for analysis.

Phenol-Camphor.—One part of phenol readily dissolves 3 parts of camphor to form a clear solution. Phenol loses its corrosive action in the presence of camphor and as such the solution is non-corrosive and is therefore used extensively for treatment of tooth-ache, skin diseases, etc. No report of poisoning from its internal administration is available in the literature. But lately two cases of fatal poisoning by taking 2 or 3 teaspoonfuls of phenol-camphor mistaken for Tinc. Opii Camphorata (Paregoric) were investigated by one of us in the Chemical Examiner's department, Calcutta. Both the patients died in about 35 minutes after taking the medicine on the same day. The stomach and a portion of the small intestine showed patches of congestion and hæmorrhage, but they were not so extensive as are usually found in cases of phenol poisoning. Both of them had convulsions before death. The following table shows the amounts of phenol and camphor extracted from the viscera and stomach contents. The figures indicate gramme per kilo of the material:

	VISCERA		STOMACH-CONTENTS	
	Phenol	Camphor	Phenol	Camphor
Case No. 1	0.55	Traces	0.25	0.56
Case No. 2	0.50	Traces	0.33	0.88

Both phenol and camphor are highly toxic and cause death by respiratory and cardiac failure, but the amounts of phenol and camphor present in 2-3 teaspoonfuls of phenol—camphor are individually not sufficient to cause death in such a short time. It is likely that each of them intensified the action of the other and their combined toxic action was sufficient to cause death. The loss of corrosive action of phenol appears to have left the mucous surfaces of the stomach and intestines in a much healthier condition and thus facilitated the absorption of both the poisons more readily than what is usually expected in poisoning by phenol.

6. Cresol and Lysol

Cresol (B.P.) is a mixture of three isomeric (ortho-meta- and para-) cresols obtained from coal tar. The mixed cresol is also known as 'trikresol'.

Extraction—Crude cresols are used in preparing lysol, which is very similar to Liquor Cresolis Saponatus B.P., by treating the former with linseed oil and KOH. To establish the presence of lysol it is necessary to prove the presence of both cresol and fatty acids. The extraction of cresols from viscera, stomach-contents, etc., is effected in the same way as is done in the case of phenol. The distillate contains cresols, some hydrocarbons (usually present with crude cresols) and traces of fatty acids (from linseed oil). Extract the distillate with ether which takes up all the three components. Evaporate the ether extract to dryness and dissolve the residue in NaOH solution. Shake the alkaline solution with petroleum ether which takes up the hydrocarbons. Acidify the alkaline solution with HCl, cresols and fatty acids are liberated. Extract with ether, evaporate the ether extract to dryness and dissolve the residue in Na_2CO_3 solution which fixes up only the fatty acids and not the cresols. Again extract with ether—the ether extract now contains only the cresols. Evaporate it to dryness and dissolve in water and test for cresols.

Tests for Cresol

(i) *Ferric Chloride Test*.—Dilute ferric chloride solution gives a bluish violet colour which disappears quickly and is followed by a dirty turbidity.

(ii) *Ware's Nitrite Test*.—Positive (see p. 448).

(iii) *Ware's Nitrite-Nitrate Test* and

(iv) *Benzaldehyde Test*.—Negative. (See p. 449).

Quantitative Determination of Cresol

Weigh out the material and proceed with the bromate-bromide method of determination as in the case of phenol. Take an aliquot portion (about 50 c.c.) of the distillate and remove the hydrocarbons and fatty acids as described before. Dissolve the final ether residue carefully in about 25 c.c. of water and transfer the solution to a glass-stoppered flask for actual titration. In the case of cresol the factor is 0.001801. Multiply the difference between the number of c.c. of N/10 bromine originally added and the number of c.c. of N/10 sodium thiosulphate required for titration, by the factor 0.001801 and the result gives the amount of cresol present in the aliquot portion of the distillate taken for examination.

N.B. It may be noted that putrefaction of protein materials may give rise to traces of volatile and non-volatile compounds some of which are of the nature of phenol and cresol and as such they give the tests described in the preceding pages. Opinions should, therefore, be guarded if any positive reaction for phenols is obtained in cases of *post mortem* materials from highly decomposed bodies.

CORROSIVE ALKALIES

Potassium, Sodium and Ammonium

The alkalies such as the hydroxides of potassium, sodium and ammonium are strongly corrosive, due to "their solvent action on protein material, their saponifying action on the lipides and their ability to extract water from the tissues." The carbonates and bicarbonates being feebly corrosive (and that is only in strong solution) are not of much medicolegal importance. Lime or slaked lime in the form of lime paste as taken with *pán* is also strongly corrosive but poisoning by lime is practically unknown except a few cases of accidental poisoning which have not proved fatal.

Since K and Na are normal constituents of animal and plant tissues and since ammonium is a decomposition product of nitrogenous organic matter, it is necessary to prove that

their hydroxides or carbonates are present in the free state and not as their neutral salts. The normal HCl of the gastric juice or the organic acids present in food materials neutralize only a small portion of the alkali but the antidotes consisting usually of vinegar, lime juice, tartaric acid, etc., always administered in cases of such poisoning, neutralize completely the alkalies and the reaction of the stomach contents may, therefore, be neutral or even acidic. As most of the alkalies are usually vomited at the early stage of the poisoning, the vomit may give a definite information about the nature of the poison used. One should not expect much from the examination of the tissues such as liver, kidney, etc., since *the absorbing surface of the stomach is practically dissolved out in most cases by the strong alkali.*

Extraction of Sodium and Potassium Hydroxides.

—The alkalies either in their own form or as salts may be extracted from the stomach tissues, stomach-contents or vomit by the following method:

Digest the material with 3 or 4 volumes of distilled water on the steam bath. Filter and evaporate the filtrate to dryness. Extract the residue with hot *absolute alcohol which dissolves the hydroxides but not the carbonates and other salts.* Evaporate the alcoholic solution to dryness and dissolve it in a small quantity of water, the solution would contain the hydroxides. The residue containing the carbonates is also dissolved in water. Both the solutions may now be tested for sodium and potassium.

Tests for Potassium

(i) *Flame Test.*—The characteristic violet flame—best seen through a cobalt glass or an indigo prism.

(ii) *Perchloric Acid Test.*—Neutralize the solution with HCl. Add a few drops of aqueous solution of perchloric acid (1:1) to about 2 c.c. of the neutral solution—a white crystalline precipitate of potassium perchlorate KClO_4 is formed. If no precipitate, concentrate the solution further and test with concentrated perchloric acid.

(iii) *Plantinic Chloride Test.*—Neutralize or slightly acidify the solution with HCl. Add a few drops of chloro-

platinic acid solution (H_2PtCl_6), commonly known as platinic chloride, a yellow crystalline precipitate of potassium chloroplatinate K_2PtCl_6 is formed which is insoluble in 80 per cent alcohol (distinction from ammoniac).

(iv) *Tartrate Test*.—Neutralize the solution with HCl, concentrate and add drop by drop strong solution of sodium hydrogen tartrate—a white granular precipitate of potassium hydrogen tartrate is formed on shaking.

(v) *Cobaltinitrite Test*.—Acidify the solution with acetic acid and add a few drops of a freshly prepared solution (0.25 g. in 2 c.c.) of sodium cobaltinitrite $Na_3Co(NO_2)_6$, a yellow crystalline precipitate of potassium cobaltinitrite is formed. Addition of an equal volume of alcohol hastens the reaction. With ammonia it also forms a similar precipitate.

Tests for Sodium

(i) *Flame Test*.—Sodium salts give an intense yellow flame. This test is not a confirmatory one as minute traces of sodium salts which are normally present in tissues, etc., impart the characteristic yellow colour to a colourless flame.

(ii) *Pyro-antimonate Test*.—Neutralize the solution by K_2CO_3 and concentrate. Add an excess of an aqueous solution of potassium hydrogen pyro-antimonate $K_2H_2Sb_2O_7$, shake and allow to stand for sometime—a white crystalline precipitate of $Na_2H_2Sb_2O_7$ is formed. It may be necessary to keep it overnight before a precipitate appears. The original solution must be neutral or slightly alkaline but never acidic. The presence of metals other than K and Na interferes with the test and they should be removed.

Quantitative Determination

Potassium—the amount of potassium may be determined from potassium chloroplatinate by the usual method of gravimetric analysis. The weight of chloroplatinate multiplied by the factor 0.1155 gives the amount of KOH present in the weight of the material taken for analysis.

Sodium—The quantitative determination of sodium is rarely necessary in chemico-legal investigations. New methods have lately been worked out for such determination for which books on analytical chemistry may be consulted if necessary.

Extraction of Ammonium Hydroxide and its Salts.

—As ammonium hydroxide and ammonium carbonate are volatile, they quickly disappear from stomach contents and other materials unless the examination is made soon after they are available. Antidotes neutralize the alkalinity and fix up the hydroxide and carbonate as non-volatile ammonium salts. If the material is distilled as it is, free ammonium hydroxide, if present, may be obtained and tested. If the residue (after distillation for free ammonia) is rendered strongly alkaline with a caustic alkali and again distilled, the ammonium salts will be decomposed and a further lot of free ammonia will be obtained. The distillates are collected in a few c.c. of water and tested for ammonia.

Tests for Ammonia

(i) Add a drop of *Nessler's reagent* to the distillate—a yellow or brown colouration or a dark brown precipitate is obtained.

(ii) Neutralize or slightly acidify the distillate with HCl and add a few drops of *chloroplatinic acid* (H_2PtCl_6) solution—a yellow crystalline precipitate of ammonium chloroplatinate is formed which is soluble in 80 per cent alcohol (*cf.*, potassium). If the precipitate is evaporated to dryness and extracted with alcohol-ether (3:1) mixture, the alcoholic solution gives on evaporation under the microscope beautiful yellow octahedral crystals of $(\text{NH}_4)_2\text{PtCl}_6$.

(iii) Add a few drops of *mercurous nitrate* solution—a black precipitate is formed. A strip of paper moistened with mercurous nitrate solution may be used for this test—it blackens on touching the distillate.

(iv) Add a small drop of a strong solution of *mercuric chloride* to the distillate on a slide—beautiful needle shaped crystals will be seen under the microscope.

Quantitative Determination

(a) *Free Ammonia*—Weigh out the finely minced material into a distilling flask and mix thoroughly with excess of absolute alcohol and some ether and then distil on a water bath at a temperature not exceeding 40°C . The free ammonia and ether are distilled over and collected in a receiver containing a known amount of N/10 H SO. The amount of ammonia is then determined by titration with N/10 NaOH.

Instead of distilling on a water bath as described above, the free ammonia may be expelled from the flask containing the material with alcohol and ether by passing a current of air by means of water pump for about an hour or more if necessary. The ether and ammonia will pass over to the receiver containing the known amount of $N/10$ H_2SO_4 .

(b) *Combined Ammonia*—The material having no free ammonia is weighed and taken in a distilling flask and rendered strongly alkaline with strong solution of $NaOH$ and distilled at boiling temperature. The distillate is collected in a known amount of $N/10$ H_2SO_4 , which is titrated with $N/10$ $NaOH$ and the amount of ammonia is calculated therefrom.

N.B. It may be noted here that the quantitative determination of ammonia is rarely of value because ammonia being highly volatile escapes from the stomach contents, vomit, tissues, etc., and on the other hand it is formed during the process of putrefaction of tissues and other protein materials. In either case, therefore, the result is likely to be erroneous.

CORROSIVE METALLIC SALTS

Zinc Chloride

This is a caustic substance. It readily dissolves proteins and, therefore, acts as a corrosive poison. As it is used in certain industries lately developed in this country, poisoning by this salt, mostly accidental and occasionally suicidal, has been reported.

As zinc is normally found in varying amounts in human tissues (10—45 mgm. per kilo), human hair (116-420 mgm.) and in foodstuffs (as much as 145 mgm.), mere detection of this metal in the tissues is of little medicolegal importance (*vide* Table next page).

Extraction of Zinc.—The general method of destruction of organic matter and extraction of metallic poisons from the tissues has been described on pp. 421-22. To the acid solution thus obtained apply the following tests for zinc:

Tests for Zinc

(i) Add some ammonium chloride (solid) and alkalinize the mixture with ammonia. Filter. Pass H_2S through the filtrate—a white precipitate of zinc sulphide is formed. The precipitate is soluble in mineral acids but insoluble in acetic acid and sodium acetate.

N.B. If iron is present even in traces, the precipitate becomes dark in colour.

(ii) Dissolve the zinc sulphide thus obtained in dilute HCl and add a few drops of $K_4Fe(CN)_6$ solution—a gelatinous white precipitate of zinc ferrocyanide $Zn_2Fe(CN)_6$ is formed. Add a few drops of bromine-water to the precipitate—a greenish yellow or yellow colour develops. Boil. A green or bluish green precipitate is formed.

(iii) *Mercury thiocyanate test*.—Neutralise the acid solution and take a drop of it on a microscope slide and evaporate almost to dryness. Add a drop of the reagent (prepared by dissolving 30 g. of $HgCl_2$ and 33 g. of ammonium thiocyanate in 50 c.c. of water at room temperature), characteristic feathery crystals of $Zn(CNS)_2 \cdot Hg(CNS)_2$ will be seen under the microscope.

TABLE

Zinc-contents of some foodstuffs. Figures indicate milligrams per kilo of fresh materials (Sylvester & Hughes)

Wheat germ ...	140—145.0	Potato ...	1.8
Wheat flour ...	6.5	Carrot ...	3.1
Whole meal flour ...	25.0	Beet root ...	7.4
Wheat bran ...	74—112.0	Apple ...	0.33
Indian tea ...	25—42.0	Egg (whole) ...	10.0
Cabbage ...	2.0—4.0	Egg (yolk) ...	23—29.0
Spinach ...	7.1	Egg (white) ...	nil.

Quantitative Determination of Zinc (Colorimetric method of Sylvester and Hughes).

Take a known amount (10–20 g. or an amount likely to contain 0.1–1.0 mgm. of zinc) of the material, oxidize by the nitric-sulphuric acid method and expel the excess of H_2SO_4 by heating on a sand bath. Treat the residue with 5 c.c. of 5 N hydrochloric acid, heat to boiling, dilute with 10 c.c. of water and boil again. After cooling transfer it into a separating funnel by rinsing with 20 c.c. of water, add 10 c.c. of 5 N ammonium acetate solution (prepared by dissolving 386 g. in a litre of water) and mix.

Add 5 c.c. of 0.15 per cent chloroformic solution of diphenylthiocarbazone (dithizone), shake vigorously, allow to separate and transfer the chloroform extract to a second separating funnel. Wash the chloroform extract by shaking it with a mixture of 6 c.c. of 5 N ammonium acetate solution, 3 c.c. of 5 N HCl and 10 c.c. of water. Allow to separate, transfer the chloroform layer to a third separating funnel and wash with 20 c.c. of distilled water. Transfer

the chloroform layer to a fourth separating funnel, leaving the wash waters in the 2nd and 3rd funnels. Again extract the liquid remaining in the 1st funnel with 5 c.c. of dithizone reagent and follow the above procedure, using the wash liquids left in the funnels from the treatment of the previous extract. If necessary, repeat the process until the liquid in the 1st funnel is completely extracted, as indicated by the colour of the reagent appearing unchanged after shaking. When the extractions and washings are completed, the whole of the extracts will have been combined in the fourth funnel.

To the combined extracts add 10 c.c. of N/2 hydrochloric acid and shake. Run off the chloroform layer and transfer the acid solution to a 100 c.c. pyrex beaker. Wash the funnel with about 10 c.c. of distilled water, adding the washings to the contents of the beaker. Re-extract the dithizone solution with another 10 c.c. of the dilute acid, and again wash the funnel with 10 c.c. of water, adding the acid extract and the washing to the liquid in the beaker.

Evaporate the contents of the beaker to dryness. Add 5 drops of pure perchloric acid and 5 drops of 100-vol. (30 per cent) hydrogen peroxide and heat to dryness on a hot plate. Repeat this process until all organic matter is destroyed and a white residue is obtained. Wash down the sides of the beaker with distilled water and again evaporate to dryness.

The zinc is determined in the residue so obtained by either of the following methods:—

(a) *For amounts of Zinc not exceeding 0.2 mgm.*—Add 0.1 c.c. of glacial acetic acid and a pinch (about 10 mgm.) of ammonium hydrogen fluoride, followed by 2 c.c. of 5 per cent freshly prepared KI solution and 2 drops of 1 per cent freshly prepared starch solution. If a blue colour appears after the addition of the starch, add a few drops of a very dilute solution (N/500) of sodium thiosulphate until the colour is just discharged. Add about 0.5 c.c. of 1 per cent $K_3Fe(CN)_6$ solution and, stirring with a glass rod, titrate with N/500 $Na_2S_2O_8$ solution (freshly prepared from N/10 solution). The titration gives better result if carried out in a dark room by artificial light, but this is not essential. The blue starch iodide colour may be adsorbed on the precipitated zinc ferrocyanide and in this case the precipitate serves as an indicator. Since 1.0 c.c. of N/500 $Na_2S_2O_8$ solution = 0.20 mgm. of zinc, the amount of zinc present in the material taken for determination is calculated accordingly.

(b) *For amounts of Zinc exceeding 0.2 mgm.*—Add 1 c.c. of water, 1 c.c. of glacial acetic acid, 1 c.c. of isopropyl alcohol, 5 drops of diphenylbenzidine reagent (prepared by dissolving 50 mgm. in 100 c.c. of pure glacial acetic acid, assisted by the addition of a few drops of dilute H_2SO_4 and warming, and filtering if necessary) and 2 drops of a 1 per cent freshly prepared solution of $K_3Fe(CN)_6$. A blue colour is obtained in the presence of zinc, and a dirty green colour if very small amounts of zinc are present (as in the blank on

reagents). Titrate the solution with dilute standard $K_4Fe(CN)_6$ solution (prepared by dissolving 3.24 g. of the pure salt in water and then making up to 200 c.c.; 10 c.c. of this solution is again diluted, at the time of titration, to 250 c.c.), stirring with a glass rod to ensure complete removal of the adherent solids from the bottom of the beaker. The titration is complete when the further addition of $K_4Fe(CN)_6$ ceases to cause a change in the colour of the solution.

N.B.—The titrated liquid is pale yellow in colour if iron is entirely absent, but the presence of very small amounts of iron will affect the final colour, and may make the detection of the end point almost impossible; for this reason, the greatest care must be taken with regard to the washing procedure, cleanliness of the beakers, etc., as described before.

Since 1 c.c. of the dilute standard $K_4Fe(CN)_6$ solution = 0.20 mgm. of zinc, the total amount of zinc present in the amount of the material taken for determination can be calculated.

N.B.—The results of this method of determination of zinc are not affected by any metal except bismuth (exceeding 2.0 mgm.) and cadmium (*Analyst*, Vol. 61, 1936).

Lately a more accurate method has been worked out by the Analytical Methods Committee (Sylvester being a member of this Committee) and described in detail in the *Analyst*, p. 304, Vol. 73, 1948. The presence of 5 mgm. of Bi per kilo does not interfere with extraction by this method.

2. Silver Nitrate

Silver nitrate or lunar caustic acts as an astringent and corrosive poison. Poisoning by $AgNO_3$ is comparatively rare in comparison with its extensive use in the arts and industries and also in medicine.

Extraction of Silver Nitrate.—Silver will be found in the acid solution after destruction of the organic matter by the $HNO_3 + H_2SO_4$ method (p. 421) as silver sulphate and nitrate but some may be found in the insoluble residue as silver chloride (chlorine from the normal tissue of food).

Tests for Silver

(i) To the acid solution add HCl or a soluble chloride—a white curdy precipitate of $AgCl$ which gradually blackens on exposure to light. $AgCl$ is soluble in excess of ammonium hydrate or sodium thiosulphate solution and also on boiling with concentrated H_2SO_4 , but insoluble in HNO_3 .

(ii) Potassium chromate (K_2CrO_4) gives a red precipitate of Ag_2CrO_4 with a neutral solution of a silver salt, insoluble in cold acetic acid but readily soluble in mineral acids. Therefore, no precipitation takes place in presence of mineral acids.

(iii) Ammonium hydrate gives a greyish precipitate of Ag_2O which is soluble in excess of ammonia. *If ammonia is not added very carefully to a dilute solution the precipitate is not observed.*

(iv) Add a little solid hexamethylene tetramine (hexamine) to a drop of a neutral solution of a silver salt on a microscope slide—monoclinic plates or needles are found under the microscope.

Quantitative Determination of Silver.—The best way of isolation of silver from the tissues and other materials is by ashing the materials at a dull red heat in a platinum capsule. Weigh out the material, acidify with strong nitric acid, dry and ash. Leach the ash with hot water to extract all soluble silver salts. Filter and leach the residue with ammonium hydrate which dissolves the chloride and phosphate of silver. Filter and digest the residue with hot concentrated nitric acid to dissolve the metallic silver, if any. Filter. Combine the three filtrates and alkalize with ammonia. Add HCl and precipitate the silver as $AgCl$. Proceed as with the gravimetric method of determination of chlorides described on p. 442. To obtain the weight of silver multiply the weight of $AgCl$ by 0.7527.

CHAPTER XXXIV

Volatile Organic Poisons and Volatile Alkaloids : Hydrocyanic Acid and Cyanides. Ethyl and Methyl Alcohols. Chloral Hydrate. Chloroform. Kerosene. Aniline. Nicotine. Non-Volatile Alkaloids. Morphine. Strychnine. Atropine. Aconitines. Cocaine.

1. VOLATILE ORGANIC POISONS

The volatile organic poisons are responsible for quite a large number of fatalities which the toxicologists are required to deal with. Those poisons which are fairly common in this country have been discussed in these pages.

1. Hydrocyanic Acid and Cyanides

Hydrocyanic acid is a most powerful poison but it is not accessible to all. Cyanides of sodium and potassium are also highly poisonous but are at the same time easily accessible on account of their extensive use in various industries. The ferro- and ferri-cyanides are positively non-toxic, for with the dilute HCl of the gastric juice at body temperature they yield only traces of HCN which are readily dealt with by the system. Certain plants, vegetables and fruits containing cyanophoric glucosides, e.g., the ordinary bamboo shoot (used as a vegetable), linseed flower, small jowar plant or millet (*Sorghum vulgare*), bitter almond, the skin of cassava or *simul alu* (*Manihot utilisima*), and certain oil seeds and beans are highly poisonous on account of the ease with which their glucosides are rapidly hydrolyzed in the stomach and free HCN is liberated in toxic doses and thus cause fatal poisoning of men and cattle in most parts of India. Quite a large number of cattle die every year by grazing in linseed and jowar fields due to inadvertence of their keepers.

Hydrocyanic acid is readily absorbed from the respiratory tract and from all mucous surfaces, e.g., from the stomach, rectum, vagina, etc., and also from the intact skin. A portion of the poison absorbed into the system is excreted

unchanged through the lungs and it is, therefore, necessary to send one of the lungs along with other viscera for detection of this poison; a portion is excreted in the urine in the form of sulphocyanides and some of it is likely to be found in viscera and stomach contents as formates, for HCN is very readily hydrolyzed to ammonium formate.

The reaction of stomach contents in HCN poisoning is always acid if no antidotes were administered, while in the case of poisoning by NaCN or KCN, it is strongly alkaline due to hydrolysis of cyanides: $\text{KCN} + \text{H}_2\text{O} = \text{KOH} + \text{HCN}$. Alkali cyanides, if exposed to air, are readily converted into carbonates by the action of CO_2 and moisture of air (see p. 238). Old samples of these cyanides may, therefore, contain quite a considerable amount of carbonates and only a small amount of cyanides. Hence suicidal attempts by taking large doses of such cyanides have been known to be unsuccessful and one of such cases was investigated in the Chemical Examiner's laboratory, Calcutta. In cases of poisoning by KCN, corrosions of stomach wall due to the action of KOH and K_2CO_3 are frequently found.

Extraction of HCN—In poisoning by HCN, free HCN may not always be detected in the organs sent for analysis due to the following reasons: (a) A portion of it combines with sulphur from decomposing proteins and is transformed into sulphocyanates, (b) a portion combines with the aldehyde group of glucose normally present in blood and other tissues, (c) a portion is hydrolyzed and converted into ammonium formate and (d) the remaining portion is lost by actual dissipation of the volatile acid from the tissues. These conditions are, however, of rare occurrence and are only possible if death is caused by the minimum fatal dose of HCN and the materials are collected from highly decomposed cadavers. In actual practice, however, it has been detected fairly readily in decomposed bodies exhumed even after several months.

Tests for HCN

(i) *Preliminary Tests with Test Papers*.—Take a small portion of the material in a flask and acidify with tartaric acid. Close with a tightly fitting cork whose lower end is

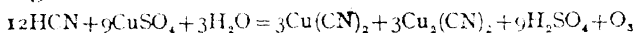
provided with a slit into which is inserted one of the test papers for detection of HCN. Now heat the flask on the water bath, HCN, if present, will impart a blue or bluish green colour to these papers. The following test papers have been found very useful in detecting even minute traces of free HCN:—

(a) *Guaiacum-Copper sulphate Paper*.—Prepare by wetting strips of filter paper first with a 10 per cent alcoholic solution of guaiacum and then in a 0.1 per cent aqueous solution of CuSO_4 and allow to dry.

(b) *Benzidine-Copper acetate Paper*.—Mix 25 c.c. of one per cent. benzidine acetate solution (prepared by heating 2.3 grms of pure benzidine acetate in 100 c.c. of water for 10—15 minutes at 80°C with constant stirring and then filtering, the clear solution being approximately 1 per cent) and 2 c.c. of 3 per cent cupric acetate solution, stir well and dip the strips of filter paper in the mixture for one minute and allow to dry. N.B. *The mixed reagent will not keep more than 15 minutes, and the papers must be used immediately after they are dry.*

(c) *Congo red—Silver nitrate Paper*.—Dip strips of filter paper in 0.05 per cent congo red solution for one minute and thoroughly dry. Dip again in 15 per cent AgNO_3 solution and dry as rapidly as possible in a dark place.

N.B. The reaction of the first two test papers depends on the formation of ozone and production of a blue compound with guaiacum or benzidine:



These tests are not specific for HCN. They indicate also the presence of NH_3 , HCl , HNO_3 , Cl , Br , H_2O_2 , etc. The negative result is, therefore, important as it rules out completely HCN and the soluble cyanides.

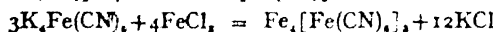
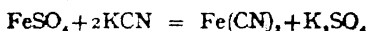
(ii) *Silver Nitrate Test*.—Proceed as with test papers but instead of closing the flask with a cork, invert carefully on the mouth of the flask a microscope slide with a small drop of AgNO_3 solution on it. Gently heat the flask on the water bath if necessary, when HCN gas produces a white

turbidity in the AgNO_3 solution. Examine under the microscope—beautiful needle shaped crystals of AgCN will be found.

(iii) *Prussian Blue Test*.—Mince the material finely, mix with 3.5 times of H_2O , acidulate with tartaric acid, and distil. Collect a few c.c. of the distillate in a receiver containing some 5 per cent KOH solution, care being taken in keeping the condenser and receiver cool by ice water, and in dipping the outlet of the condenser under KOH solution in the receiver. Take 2-3 c.c. of the alkaline distillate and proceed as described in p. 237 (Chapter XIX).

N.B. Too much HCl retards the formation of Prussian blue. The optimum concentration of HCl should be about 0.5 normal. *In concentrated HCl , Prussian blue dissolves and gives a yellow colour.* "The Prussian blue formation does not depend upon a simple instantaneous ionic reaction. It is a time reaction which is retarded or prevented by excess of ferric and other ions" (Vorlander). In an apparently negative reaction, if the test tube is kept overnight a flocculent precipitate of Prussian blue may settle at the bottom.

The following reactions take place during the process of formation of Prussian blue:



(iv) *Sulphocyanate Test*.—To about 3.5 c.c. of the alkaline distillate, add about 1 c.c. of yellow ammonium sulphide solution. Evaporate the mixture almost to dryness on a water bath. Dissolve the residue in a little water and acidify with dilute HCl or HNO_3 . Warm and filter through double filter paper and repeat the filtration several times till the filtrate is clear. Add a few drops of very dilute FeCl_3 or ferric alum solution—a reddish to blood red colour, due to formation of ferric sulpho-cyanate, $\text{Fe}(\text{CNS})_3$, develops according to the concentration of HCN . The red colour is discharged by mercuric chloride solution.

(v) *Vortman's Nitroprusside test*.—To about 3.5 c.c. of the distillate, add a small crystal or a few drops of a freshly

prepared potassium nitrite solution, and 2-4 drops of FeCl_3 solution and sufficient dilute H_2SO_4 to impart to the mixture a bright yellow colour. Heat it to boiling, allow to cool and add dilute ammonia to precipitate the excess of iron, and filter. Add to the filtrate a few drops of very dilute ammonium sulphide solution—a violet colour appears which gradually changes to blue, green and then to yellow. This test is due to the formation of potassium nitro-prusside $\text{K}_2[\text{Fe}(\text{NO})(\text{CN})_5]$. Hydrocyanic acid, if present, in very small amounts, gives a bluish green to greenish yellow colour.

Quantitative Determination

(a) *Gravimetric*.—Weigh out a portion of the material in a distilling flask, acidify with tartaric acid and distil with steam as described in p. 7, the distillate being received in a conical flask containing about 20 c.c. of 10 per cent AgNO_3 solution acidified with HNO_3 . Continue the distillation till white precipitate of AgCN is formed in the receiving solution. Filter through a tared filter paper, wash the residue with water till the washing is free from AgNO_3 . Dry the filter with residue at 100°C to a constant weight. The difference in weight between the filter paper plus residue and the original weight of the filter paper gives the amount of AgCN formed from HCN present in the quantity of the material taken for estimation. Multiply this weight by the factor 0.2018 to obtain the amount of HCN, or by 0.496 to obtain the amount of KCN; or incinerate the dry filter with AgCN in a tared crucible and weigh. The weight of silver thus obtained multiplied by the factor 0.2505 gives the weight of HCN.

(b) *Volumetric (Volhard's Method)*.—Collect the distillate in a receiver containing N/10 KOH solution. Add drop by drop with constant shaking, a known excess of N/10 silver nitrate solution, acidify with HNO_3 , shake vigorously for a few minutes. Dilute up to a definite volume in a measuring flask and filter through a dry filter. Take an aliquot portion and titrate with N/10 KCNS solution using ferric alum as an indicator which forms the reddish brown ferric thiocyanate. The amount of N/10 AgNO_3 added minus the amount of N/10 KCNS required for titration represents the amount of AgNO_3 taken up by the cyanide. Each c.c. of N/10 AgNO_3 is equivalent to 0.0027 g. of HCN or 0.00651 g. of KCN.

2. Alcohol, Ethyl Alcohol

Intoxication due to excessive drinking proves fatal in many cases and such fatalities appear to be fairly common. Apart from fatal cases, cases of drunkenness are also of medicolegal importance. Purves-Stewart gives the following definition of drunkenness: 'A drunken person is one who has taken alcohol in sufficient quantity to poison the central

nervous system producing a temporary disorder of the faculties so as to render him unable to execute the occupation in which he was engaged at the time, thereby causing danger to himself or to others".

It has been proved that the toxic effects of alcohol depend largely upon its concentration in the blood, brain and other organs. As alcohol is absorbed very quickly and diffuses quite rapidly throughout the system, its concentration in the blood, cerebrospinal fluid, and other tissues of the body is practically the same at any particular moment after its absorption. The concentration of alcohol in the blood attains a maximum value about $1\frac{1}{2}$ hours after drinking and falls at the rate of about 12 mgms. per hour per 100 g. of blood. Its elimination through the urine bears a definite relation to its concentration in the blood. It has been found that the ratio of *alcohol in the urine to alcohol in the blood* is surprisingly constant and is of the order of 1:35 one hour after its consumption.

An approximate correlation between the results of blood and urine analysis and the degree of drunkenness has been worked out by different workers in the West and is as follows:—

80 mgms. and downwards per	100 c.c. of blood—	No indication of intoxication.
160 mgms. per 100 c.c. of blood—	Most cases show signs of moderate intoxication e.g., mental confusion diminution of control, disorders of coordination, etc.	
200 mgms. per 100 c.c. of blood—	Intoxication well marked with psychical and speech disorders and unsteady gait.	
350 mgms. and upwards per—	100 c.c. of blood—	Inability to stand or walk, stupor; coma with fatal result.

200 mgms. per 100 c.c. of the	urine—	Moderate intoxication.
360 mgms. per 100 c.c. of the	urine—	Definite drunkenness as described above.

The above figures indicate that quantitative determination of alcohol in the blood or in the urine is of great medicolegal

importance in cases of suspected alcoholism. In fatal cases of poisoning by alcohol any post mortem material will reveal the presence of alcohol.

Extraction of Alcohol—The material finely minced is mixed with sufficient water to give a consistency of gruel, acidified with tartaric acid and submitted to ordinary distillation or steam distillation—the tip of the condenser being dipped in a little distilled water in the receiver. With this distillate the following tests are carried out:

Tests for Alcohol

(i) *Iodoform Test* (for crystals of iodoform see Fig. 45) and (ii) *Ethyl acetate Test*.—See tests for Ethyl Alcohol on page 89 (Chapt. VIII).

(iii) *Ethyl Benzoate Test*.—Shake a few c.c. of the distillate with a drop or two of benzoyl chloride and then add drop by drop 10 per cent NaOH solution to render the mixture strongly alkaline. On warming, the irritating odour of benzoyl chloride disappears and the sweet fruity odour of ethyl benzoate appears. Methyl alcohol also gives this test but it does not give the iodoform test.

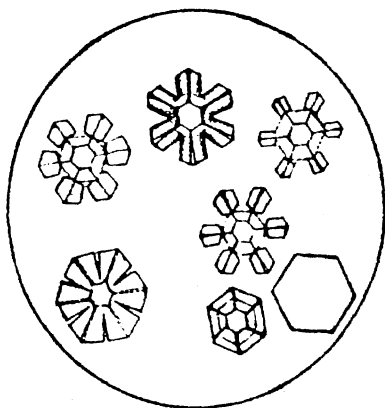


Fig. 45

(iv) *Sulphomolybdic Acid Test*.—Prepare sulphomolybdic acid reagent by dissolving by heat one gram of molybdic acid in about 25 c.c. of concentrated H_2SO_4 . Take about 2 c.c. of this hot reagent in a test tube and layer 2-3 c.c. of the distillate on the top of the reagent—a deep blue ring appears at once at the junction of the two liquids. On shaking, the whole mixture becomes deep blue.

N.B. This test is very sensitive and is negative with acetone, acetaldehyde and dilute solution of methyl alcohol.

Strong solution of methyl alcohol gives only a light blue colouration after several minutes.

Quantitative Determination of Alcohol

There are several methods (physical and chemical) for the determination of alcohol but *Southgate's semi-micro method* is the best and is not open to the objections and fallacies pointed out in other methods. Besides, it has the advantage of being used with a small amount of the material (1-2 c.c.) and is therefore applicable to cases under the Traffic Acts in which 1-2 c.c. of blood or urine is taken from the person charged of drunkenness.

Weigh out about 100 grams of finely minced viscera or stomach contents in a distilling flask. Mix with plenty of water to give it a consistency of gruel, acidify with tartaric acid and distil with steam—the condenser and the receiver being kept cool with ice water and the outlet of the condenser being dipped in a little water in the receiver. Continue the distillation until about 2 c.c. is obtained for each gram of the material taken. As interfering substances such as acetone bodies, aldehydes, etc., are likely to be present in the distillate, add to it 5 c.c. of 10 per cent NaOH and 3 c.c. of 10 per cent AgNO_3 solutions and distil again (steam distillation not necessary this time). Measure carefully the total amount of this distillate and pipette off accurately (with a calibrated pipette) just 2 c.c. for actual determination.

Take in the inner tube of the apparatus (Fig. 46) 10 c.c. of N/10 potassium dichromate solution and 10 c.c. of concentrated

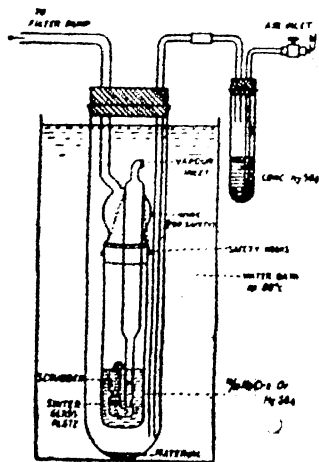


Fig. 46

H_2SO_4 and in the outer tube 2 c.c. of the distillate. Connect the different parts of the apparatus and start the air current and then immerse the apparatus in the trough of water maintained at 80°C . The alcohol volatilizes and is carried by the current of air through the $\text{K}_2\text{Cr}_2\text{O}_7$ and H_2SO_4 mixture where it is broken up into fine bubbles by the sinter-glass plate and is completely absorbed and oxidized. Continue the passage of air current for about an hour or until the material is completely evaporated. Transfer the dichromate solution carefully into a litre conical flask, wash the apparatus several times with copious water so that the final volume may be about 400-500 c.c. Now add 5 c.c. of a freshly prepared 0.4 N solution of KI (51.2 g. per litre) and a few drops of 1 per cent starch solution.

Titrate the mixture with N/10 sodium thiosulphate solution (freshly

standardized) until the deep blue colour just disappears and a pale green colour of $\text{Cr}_2(\text{SO}_4)_3$ appears. From the number of c.c. of thiosulphate required for titration, the amount of alcohol is calculated.

The difference between the amount of $\text{N}/10 \text{ K}_2\text{Cr}_2\text{O}_7$ taken and the amount of $\text{N}/10 \text{ Na}_2\text{S}_2\text{O}_3$ used is the amount of dichromate (X) reduced by alcohol. As each c.c. of $\text{N}/10 \text{ K}_2\text{Cr}_2\text{O}_7$ is equivalent to 1.15 mgm. of alcohol, the concentration of alcohol in the distillate (mgm. per 100 c.c.) is, therefore, $X \times 1.15 \times 50$ mgm.

As this apparatus is expensive and delicate, it may be replaced by a simple arrangement as shown in Fig. 47. In this simplified apparatus the current of air should be slower to prevent splashing, and the time required for oxidation of alcohol should be correspondingly longer.

For elimination of the interfering volatile substances such as aldehydes, ketones, phenols and acids from the blood or urine, a third tube charged with

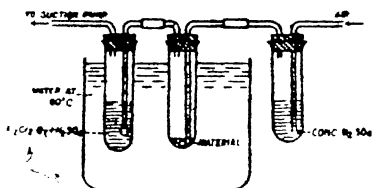


Fig. 47

10 c.c. of saturated HgCl_2 solution and 10 c.c. of saturated NaOH solution may be interposed between the tube containing the material for examination and the tube containing the dichromate mixture.

N.B. As alcohol and these interfering substances are known to be normally present to the extent of about 11 mgms. per 100 c.c. of blood (Bagchi and Ganguli) in Indians this normal value may, therefore, be deducted from the result obtained in samples of blood or urine and the extra tube for purification by treatment with alkaline mercuric oxide may be omitted.

The following analytical results show the amount of ethyl alcohol found in various post-mortem materials in a case of fatal poisoning by alcohol received from the American Army Hospital, Calcutta:—

Urine	431.5	mgms. per 100 c.c.
Heart blood	368.0	" " " "
Cerebrospinal fluid	333.5	" " " "
Stomach contents	399.5	" " " "

3. Methyl Alcohol

Poisoning by methyl alcohol has, until recently, been unknown in this country. Cases of fatal poisoning by bazar methylated spirit are known but they are actually cases of poisoning by ethyl alcohol since methyl alcohol or wood

spirit is not used in India in denaturing spirit for domestic use (see p. 86).

Drinking methyl alcohol is definitely harmful to the eye, and doses as low as 15 c.c. (about half an ounce) have been known to cause optical atrophy and blindness. Large doses prove fatal; lately, the stomach contents of a soldier who died in an American Military Hospital near Calcutta gave 6.6 per cent of methyl alcohol and no ethyl alcohol.

The diagnosis of methyl alcohol poisoning should be made, during life, by the examination of the urine for formic acid, while after death, both formic acid and methyl alcohol may be present in the blood and other tissues of the body. As the kidneys normally eliminate 0.251 gram of formic acid in 24 hours (Autenrieth), a quantitative analysis of the urine for formic acid (see below) is essential before any opinion is given in cases of poisoning (non-fatal) by methyl alcohol. The greater toxicity of methyl alcohol is due to the difficulty of its oxidation and to the production of formaldehyde and formic acid in the tissues. Its elimination is also slow; a large dose of methyl alcohol requires more than a week for elimination. It shows cumulative effects.

Extraction and Detection—Distil the material as in ethyl alcohol and submit the distillate to various tests as described under methyl alcohol (see pp. 78-80).

Detection of Methyl Alcohol in presence of Ethyl Alcohol

Take 5 c.c. of the distillate, add 2 c.c. of a solution of KMnO_4 in H_3PO_4 (prepared by dissolving 3 g. of KMnO_4 in a mixture of 15 c.c. of syrupy phosphoric acid and 70 c.c. of water and then making up to 100 c.c.); set aside for 10 minutes and add 2 c.c. of 5 per cent solution of oxalic and sulphuric acids (prepared by dissolving 5 g. of oxalic acid in a cooled mixture of equal volumes of conc. H_2SO_4 and water); to the colourless solution add 5 c.c. of Schiff's reagent (prepared according to B.P. method), and set aside for 10 minutes; a pink colour is produced if methyl alcohol is present (B.P. 1932).

Quantitative Determination of Methyl Alcohol

The steam distillation is continued till complete recovery of the alcohol, either pure methyl alcohol or methyl mixed with ethyl alcohol, is effected. Transfer 10 c.c. of the distillate to a 25 c.c. volumetric flask and add 1 c.c. of 5 per cent ethyl alcohol. Add 1 c.c. of 1 per cent KMnO_4 solution and then 1 c.c. of phosphoric acid solution (prepared by diluting 25 c.c. of 85 per cent phosphoric acid with water to 100 c.c.). Allow the mixture to stand for 1 hour with occasional shaking. Add 1 c.c. of 5 per cent oxalic acid solution. When the mixture is colourless, add 2 c.c. of H_2SO_4 solution (prepared by diluting 30 c.c. conc. H_2SO_4 with water to 100 c.c.). Then add 5 c.c. of Schiff's reagent (see p. 80). Mix by inverting the flask. After 3 hours compare in a colorimeter against a standard treated *exactly in the same way at the same time*.

This method is quite satisfactory for a concentration varying from 1 mg. to 2.5 mg. of methyl alcohol per 10 c.c. (100-250 parts per million). It is therefore desirable to arrange quantitative determinations within this range, although as little as 0.05 mg. in 10 c.c. (5 parts for million) may be detected qualitatively by this method.

Quantitative Determination of Formic Acid

The determination is based on the reduction of KMnO_4 solution in the presence of an alkaline carbonate. The steam distillation is continued for several hours to separate the formic acid from the urine or from other materials. Take an aliquot part (100 c.c.) of the distillate and render it distinctly alkaline with sodium carbonate, warm and add an excess of N/10 KMnO_4 solution and keep it warm for 5 minutes. Acidify with sulphuric acid, add a measured volume of N/10 oxalic acid solution, sufficient to dissolve all the precipitate of manganese hydroxide and react with the unreduced permanganate solution. Titrate the excess of oxalic acid with N/10 KMnO_4 solution. Also titrate the same volume of N/10 oxalic acid as used in the test as a blank. The difference between the amount of KMnO_4 used in the blank and the amount used in the actual determination is equivalent to the amount of N/10 KMnO_4 solution required to oxidise the formic acid. One c.c. of N/10 KMnO_4 = 0.002301 gm. of formic acid.

4. Chloral Hydrate

Poisoning by chloral hydrate has lately been of frequent occurrence in Calcutta. The victims are usually the public women addicted to drinking. The drug is administered with beer for purposes of robbing.

Chloral hydrate is rapidly and completely decomposed into chloroform and a formate by an alkali. During the putrefactive processes the tissues become alkaline in reaction and

any chloral hydrate retained there undergoes the same chemical changes and is thus lost for purposes of detection. It may, however, be recovered in some cases if the tissues are only slightly alkaline.

In all cases of poisoning by chloral hydrate (fatal or non-fatal), it is excreted in the urine mostly as *urochloralic acid* (trichloroethyl-glycuronic acid) and partly as unchanged chloral. The detection of urochloralic acid in the urine is, therefore, the most reliable method for diagnosis of chloral hydrate poisoning, particularly in view of the fact that fresh samples of urine undergoing no ammoniacal decomposition are rarely available for examination.

The urine in chloral hydrate poisoning reduces Fehling's solution *but is distinguished from glycosuric urine by its distinct laevorotatory property.*

Extraction of Chloral Hydrate.—Take the urine or the finely minced tissue in a distilling flask and add about half its weight of 20 per cent solution of H_3PO_4 and steam distil. To the distillate apply the following tests for chloral:

Tests for Chloral Hydrate

(i) *Phenyl Isocyanide Test.*—See p. 67. This test is also given by chloroform, bromal, bromoform, iodoform, trichloroacetic acid, etc.

(ii) *Resorcin Test.*—To 2-3 c.c. of the distillate add a pinch of resorcinol (about 0.1 g.) and 1 c.c. of 15 per cent NaOH solution and heat to boiling, a yellowish red to red colour. This test is also given by chloroform and other compounds.

(iii) *Naphthol Test.*—Dissolve about half a gram of alpha- or beta- naphthol in strong NaOH solution (33 per cent), add to it about 2 c.c. of the distillate and heat, a fine blue or bluish green colour appears which changes to greenish brown on keeping. If the blue liquid is acidified a pink to brick-red coloured precipitate is produced. This test is also given by chloroform and other compounds.

(iv) *Pyridine Test.*—Mix 2 c.c. of pyridine with 2 c.c. of NaOH solution (10 per cent), heat to boiling and then add 1-2 c.c. of the distillate, a pink or deep red colour is

produced. This test is very delicate but is positive with chloroform and other compounds.

(v) *Reduction Test*.—To 2-3 c.c. of the distillate add 1 c.c. of ammoniacal silver solution and gently heat, a shining mirror of metallic silver is formed. In the case of chloroform no mirror is formed but on boiling blackening takes place.

(vi) *Nessler Test*.—To 2-3 c.c. of the distillate add a few drops of Nessler's solution, a yellow to reddish brown precipitate which turns grey or black. With chloroform no such precipitate is formed.

(vii) *Phloroglucinol Test*.—To 1 c.c. of the distillate add 4 drops of a saturated solution of phloroglucinol and 1 c.c. of 20 per cent Na_2CO_3 solution and allow to stand. After about half an hour, a pinkish violet colour appears which changes to orange, red and deep red. If very small quantities of chloral are present, the colour change proceeds up to the stage of orange. Chloroform does not give this test, it shows only a slight violet colouration, but aldehydes give a reddish colour which may, however, be distinguished by the Schiff's reagent.

Detection of Urochloralic Acid

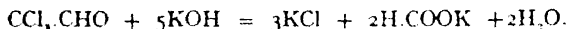
Evaporate the whole amount of urine available for analysis to a syrupy consistency on a water bath. Acidify with dilute H_2SO_4 and extract several times with an alcohol and ether (1:3) mixture. Draw off the alcohol-ether layer and distil off the solvent. Neutralize the residue with KOH and evaporate to dryness. Extract the dry residue by boiling with 90 per cent alcohol, filter and precipitate the potassium urochloralate by the addition of ether. Filter off the chloralate and wash with ether and alcohol. Again boil with absolute alcohol and filter hot. Dissolve it in a little water and acidify with dilute HCl. Shake out this solution with an ether-alcohol (8:1) mixture and draw off the solvent. Distil off the solvent and treat the residue with moist silver oxide until no more chloride precipitates. Filter off the AgCl and remove the excess of silver from the filtrate by means of H_2S . Filter and evaporate to a syrup and allow to stand overnight when free urochloralic acid

separates out in the form of silky needle-shaped crystals (m.p. 142°C).

On refluxing these crystals with 7% HCl for 2-3 hours, the acid is hydrolyzed into glycuronic acid and trichloroethyl alcohol. If this mixture is distilled, glycuronic acid gives furfural which is identified by its producing an intense red colour with a solution of aniline acetate.

Quantitative Determination of Chloral Hydrate

The steam distillation as described under extraction is continued for 18-20 hours to ensure complete recovery of chloral hydrate. Render the distillate distinctly alkaline with alcoholic KOH solution and reflux for 5-6 hours (which converts chloral into KCl and potassium formate):



Concentrate the mixture on the water bath. Acidify with dilute HNO_3 and add excess of AgNO_3 solution to precipitate the chloride as AgCl . Filter, wash, dry and weigh the AgCl in the usual way of gravimetric analysis. Multiply the weight of AgCl by the factor 0.3845 and the result would give the amount of chloral hydrate originally present in the quantity of the material taken for estimation.

5. Chloroform

Poisoning by chloroform takes place mostly from its inhalation and occasionally from ingestion by mouth. It is excreted mainly through the lungs and only small amounts escape in the urine, perspiration and milk. For detection of chloroform the organs of special value are the brain, lungs, liver and the stomach.

As chloroform is one of the products of decomposition of chloral hydrate, its presence in the tissues indicates poisoning either by chloroform or by chloral. It is, therefore, essential to find out the reaction of the material before proceeding with the extraction of CHCl_3 from the materials received for analysis. If the reaction is neutral or acid, the presence of chloroform is, *ipso facto*, due to poisoning by chloroform and not by chloral hydrate, but if the reaction is alkaline which favours the decomposition of chloral into chloroform and formates, the possibility of poisoning by chloral hydrate has to be ruled out before one gives his opinion in favour of chloroform poisoning, and for this purpose a special examination should be made for detection of formates.

Extraction and Tests.—Acidify the material and distil in steam. To the distillate apply the tests described under chloral hydrate (p. 472). In the case of chloral hydrate poisoning, the tests for both CHCl_3 and CCl_3CHO may be positive but in chloroform poisoning the tests for chloral must be negative.

Quantitative Determination of Chloroform

Take 100 grams of the finely minced material in a distilling flask, add 450 c.c. of alcohol and 25 c.c. of 5 per cent alcoholic tartaric acid solution and distil on a water bath. The outlet of the condenser is fitted up with an adapter the end of which is below the surface of about 10 c.c. of alcohol taken in the receiver. Collect only 200 c.c. of the distillate which would contain the whole amount of CHCl_3 present originally in the material. Transfer the distillate to a conical flask, add 10 c.c. of 10 per cent alcoholic NaOH solution and reflux for 1-2 hours. Cool, neutralize with H_2SO_4 using phenolphthalein as an indicator, add 0.5 c.c. of a 5 per cent aqueous solution of potassium chromate and titrate the chlorides with $\text{N}/10$ AgNO_3 solution. Each c.c. of $\text{N}/10$ AgNO_3 is equivalent to 2 mgms. of chloroform and 2.8 mgms. of chloral hydrate.

6. Paraffins—Kerosene

Poisoning by ingestion of kerosene is not uncommon and even cases of fatal poisoning, mostly accidental or suicidal, are also known. Workers exposed to vapours of kerosene suffer from chronic poisoning by inhalation and those required to handle petrol or kerosene in petroleum and allied industries suffer from acute skin lesions due to the irritant action of the hydrocarbons, particularly those containing higher percentage of sulphur compounds.

The stomach and its contents should be selected for examination if poisoning be caused by ingestion, while the lungs, brain and other organs would reveal the presence of kerosene if death is due to inhalation.

Extraction of Kerosene—Distil the material with steam. Add excess of benzene to the distillate. Shake and separate the benzene layer in the usual way. Evaporate the solvent on a water bath and test the residue for kerosene.

Tests.—Produces grease spot on paper, possesses a characteristic smell and is not saponifiable by alcoholic potash. If the quantity isolated is sufficient, its boiling

point (150° — 300°C) and other physical properties may be determined.

Quantitative Determination

After evaporation of the solvent as described above, the residue may be weighed and the weight thus obtained represents the amount of the paraffin in the known amount of the material taken for examination.

7. Aniline and its Derivatives

Aniline and its derivatives are used extensively in various industrial processes and they gain access to the body through the lungs, skin and the mouth. Fatal poisoning by aniline has not been reported in this country but fatalities from the absorption of aniline derivatives through the skin are on record. The latest fatal case was one from Kidderpore Dock (1939) where two coolies happened to sprinkle paranitraniline over their body and in about three hours both of them became unconscious and one of them died of aniline poisoning on the third day. The use of aniline dyes in the inner sole of shoes and boots and in clothes has been the source of aniline poisoning in other countries.

Aniline produces methæmoglobin in blood and, therefore, reduces the capacity of blood to retain oxygen. In aniline poisoning the amount of available oxygen is reduced to 5—10 volumes instead of 15—20 of normal blood.

It is partly changed in the human system to aniline black. At the height of aniline poisoning, "blue black granules may be seen in every drop of blood and also in the urine." Aniline is oxidized in the tissues to *p*-aminophenol which combines mostly with sulphuric acid to form *p*-aminophenyl sulphuric acid $\text{HO.SO}_2.\text{O.C}_6\text{H}_4.\text{NH}_2$, and partly conjugated with glycuronic acid. The former subsequently combines with alkalies and is eliminated through the kidneys as an alkali salt. The urine in aniline poisoning has normally a very dark colour and reduces Fehling's solution (due to presence of conjugated glycuronic acid).

Extraction of Aniline.—As aniline is a base, the material suspected to contain aniline should be rendered strongly alkaline and distilled with steam. Alkalinize the distillate with NaOH and shake out with ether, separate the ether layer and evaporate ether on a water bath when

brownish drops of aniline, if present in excess, will form the residue. Dissolve residue in water and test the solution for aniline.

Tests for Aniline

(i) *Dichromate Test*.—Take a little of the original residue (before being dissolved in water) in a porcelain basin, add 5 or 6 drops of conc. H_2SO_4 and one drop of saturated solution of $\text{K}_2\text{Cr}_2\text{O}_7$, in a few minutes the edge of the mixture begins to show a pure blue colour. Add a few drops of water—the whole becomes uniformly blue.

(ii) *Phenyl Isocyanide Test*.—See chloral hydrate and chloroform (p. 472).

(iii) *Hypochlorite Test*.—Add a few drops of sodium hypochlorite or freshly prepared solution of bleaching powder to the solution, when a purple or violet-blue colour will appear which changes to reddish brown or dirty red. Add a few drops of a dilute aqueous solution of phenol containing some ammonia to the violet or dirty red solution, a blue colour appears (*Indophenol reaction*).

(iv) Add a few drops of bromine water to the solution—a flesh coloured precipitate of tribromaniline separates, m.p. 118° .

Quantitative Determination of Aniline

Aniline may be estimated volumetrically by the following modification of the bromate-bromide method: Take 25 c.c. of the distillate (containing approximately 0.01 M solution of aniline or less) in a stoppered flask, add 25 c.c. of N/10 potassium bromate solution, 0.5 g. of KBr and 5 to 10 c.c. of 4N sulphuric acid. Stopper the flask and allow to stand for 5-10 minutes. Add 1 g. of KI and titrate with N/10 sodium thiosulphate solution, stirring thoroughly during titration. Add a few drops of 1 per cent starch solution towards the end of the titration and a few c.c. of chloroform which prevents the absorption of some iodine by tribromaniline. One c.c. of N/10 bromate is equivalent to 0.001555 g. of aniline.

Aniline may also be estimated by direct titration with standard bromine solution using indigo carmine as the indicator. The indicator becomes colourless with an excess of bromine (Kolthoff & Furman).

II. VOLATILE ALKALOIDS

The volatile alkaloids do not contain any oxygen in their molecule and are mostly liquid with characteristic odours.

They are strongly alkaline in reaction and are readily distilled without undergoing any change during distillation.

Nicotine and coniine are two liquid volatile alkaloids of toxicological importance. Poisoning by coniine has so far not been reported in this country but poisoning by tobacco (nicotine) is occasionally met with although fatal cases are comparatively few. Infanticide by tobacco leaf introduced into the mouth of a new born baby is not uncommon in some parts of India.

Nicotine, $C_{10}H_{14}N_2$

Nicotine, the most toxic alkaloid of tobacco *Nicotiana tabacum* (N.O. Solanaceæ), is found in all parts of the tobacco plant but particularly in the leaf, which contains as much as 6 per cent of the alkaloid, in combination with malic and citric acids. It is an oily colourless liquid, soon turning yellow and brown on exposure to air and in course of time becoming resinous. It is freely soluble in water, alcohol, ether, petroleum ether, amyl alcohol, benzene and chloroform. Aqueous and alcoholic solutions of nicotine are alkaline to litmus but not to phenolphthalein. It is laevorotatory but its salts are dextrorotatory. It is a strong diacid base and is a derivative of both pyridine and pyrrolidine with the chemical name of β -pyridyl- α -n-methylpyrrolidine. On oxidation with $K_2Cr_2O_7$, $KMnO_4$ or HNO_3 it is converted into nicotinic acid.

It is the most important ingredient of horticultural insecticides used in killing insects infesting plants. The "Hookah-water", which contains varying proportions of nicotine, is used in this country for the same purpose. Commercial nicotine used in manufacturing insecticides is frequently adulterated with ammonia and pyridine, and as such its nicotine concentration varies from 30 to 95 per cent.

The nicotine content of different forms of tobacco used in smoking varies considerably. Pipe tobacco contains 0.5 to 0.8, cigars 0.8 to 2.9 and cigarettes 1.0 to 2.9 per cent of nicotine. It has been calculated that the nicotine in the puffed smoke from a cigarette averages to 5 mg. for each cigarette. If the smoking of cigarettes is continued for one hour the maximum amount of nicotine retained in the

body is calculated as 36 mg. per hour in inhaling and 27.5 mg. in puffing (Webster). The tobacco smoke contains, besides nicotine, other compounds such as CO_2 , CO, HCN, H_2S , and pyridine. A man smoking continuously for an hour may have his hæmoglobin saturated with 10 to 20 per cent of CO. The minimum fatal dose of nicotine is believed to be 60 mg.

Extraction of Nicotine.—If nicotine or tobacco is swallowed, it is found in appreciable quantities in stomach contents and in the urine and to a smaller extent in most of the internal organs. The extraction is carried out in the following way:

Mix a portion of the material (except urine and stomach wash) with excess of water and render it strongly alkaline with NaOH solution and distil in steam collecting the distillate in 5–10 c.c. of 10 per cent HCl. Alkalinize the distillate and extract with ether. Evaporate the extract to dryness at room temperature. The residue may now be tested for nicotine, first, by general alkaloidal reagents to establish the presence of an alkaloid and then by its specific tests to establish the identity of the alkaloid. This is in fact the usual procedure for detection of all alkaloids.

General Tests for Alkaloids

The following *general alkaloidal reagents* are commonly employed in toxicological analysis for detection of alkaloids:

(1) *Mayer's reagent* (Potassium mercuric iodide).—Prepared by dissolving 1.357 g. of HgCl_2 and 5 g. of KI in 100 c.c. of water (N/10 solution). It gives a white or yellowish precipitate with acid solutions of most alkaloids. In the presence of acetic acid or alcohol, it does not act satisfactorily.

Half of this strength, i.e., 0.678 g. of HgCl_2 and 2.5 g. of KI in 100 c.c. of water (N/20 solution) may also be used.

(2) *Dragendorff's reagent* or *Kraut's reagent* (Potassium bismuthous iodide).—Prepared by dissolving 80 g. of bismuth subnitrate in 200 c.c. of conc. HNO_3 and pouring this solution slowly and with constant stirring into a concentrated solution of KI (272 g. KI in about 250 c.c. of

water) and then filtering after 24 hours and making up to one litre with distilled water.

The original method of Kraut is to dissolve the subnitrate in 200 c.c. of dilute nitric acid (sp. gr. 1.18 i.e. 30 per cent HNO_3) and then adding this solution to KI solution in the usual way, when KNO_3 crystallizes out. The clear solution is decanted off after several days and then made up to one litre.

The reagent acts best in the presence of sulphuric acid and gives an orange coloured precipitate but does not act in the presence of ether or amyl alcohol.

(3) *Wagner's reagent* (Iodo potassium iodide solution). Prepared by dissolving 5 g. of iodine and 10 g. of KI in 100 c.c. of water. It gives a brown precipitate and acts best in the presence of HCl.

(4) *Silicotungstic Acid* ($\text{SiO}_2 \cdot 12\text{WO}_3 \cdot 2\text{H}_2\text{O}$).—This acid or its sodium salt in 10 per cent aqueous solution is a very delicate alkaloidal reagent particularly for nicotine. It gives a white precipitate.

(5) *Sonnenschein's reagent* (Phosphomolybdic acid).—Prepared by acidifying a warm solution of Na_2HPO_4 with HNO_3 and adding an excess of ammonium molybdate solution. The yellow precipitate is separated, washed with water, acidified with HNO_3 and dissolved in a hot solution of Na_2CO_3 . The solution is evaporated to dryness and ignited at low red heat till all the ammonium salts are volatilized. The residue is moistened with HNO_3 and again ignited. This product, sodium phosphomolybdate, is dissolved in 10 times its weight of a mixture of 1 volume of strong HNO_3 (sp. gr. 1.42) with 9 volumes of water.

It acts best in the presence of sulphuric acid and gives a yellow precipitate which may turn greenish or bluish on keeping due to reduction of molybdic acid to molybdic oxide.

(6) *Phosphotungstic Acid*.—Prepared by dissolving 100 g. of sodium tungstate and 70 g. of Na_2HPO_4 in 500 c.c. of water and acidifying the solution with HNO_3 .

(7) *Picric Acid*.—A saturated aqueous solution of picric acid is used as an alkaloidal reagent. It gives a yellow crystalline precipitate.

(8) *Chloroplatinic Acid*.—A 5 per cent solution of chloroplatinic acid (platinic chloride) is used as an alkaloidal reagent which gives a yellow crystalline precipitate.

(9) *Mercuric Chloride*.—A five per cent solution is used.

(10) *Marmé's Reagent*—consists of 3 gms. of cadmium iodide, 6 gms. of KI in 18 c.c. of water.

N.B. It may be noted here that these so-called *alkaloidal reagents* do not produce precipitates exclusively with alkaloidal salts but also with various other substances such as proteins, albumoses, peptones, creatinine, and the purine bases such as caffeine, adenine, guanine, xanthine, and hypo-xanthine. Most of these reagents also give precipitates with ammonia and its simple derivatives, such as the amines, etc. This is the reason why they are also called *reagents for "nitrogen bases."*

Tests for Nicotine

(i) *Alkaloidal Reagent*.—The following tests may be conveniently carried out on a microscope slide :

(a) *Mayer's reagent*.—Add a drop of it to the acid solution—a yellowish white amorphous precipitate is formed at once which becomes resinous afterwards.

(b) *Dragendorff's reagent*.—Add a drop of it to the solution acidulated with sulphuric acid—an orange coloured amorphous precipitate is formed at once.

(c) *Phosphomolybdic Acid*.—It gives a yellowish white amorphous precipitate in acid solution.

(d) *Silicotungstic Acid or Potassium silicotungstate*.—It is a sensitive reagent for nicotine. Take a drop of the distillate on a slide, acidify with HCl, and add a drop of the aqueous solution of silicotungstic acid—a white precipitate or cloudiness is produced.

(ii) *Crystallization Tests*.—Take a drop of nicotine on a slide and add a drop of conc. HCl and allow to evaporate over conc. H_2SO_4 in a desiccator for some time—an amorphous varnish-like deposit appears which changes to a crystalline mass. This test differentiates nicotine from coniine which gives at once a crystalline precipitate instead of the varnish-like mass.

(iii) **Roussin's Test.**—To an ethereal solution of nicotine (1:100), add some ethereal solution of iodine—a brownish-red amorphous precipitate is formed which, after standing for some hours, changes to a crystalline mass of ruby-red needle-shaped crystals known as "Roussin's crystals". With more dilute solutions, the amorphous stage of the precipitate may be absent.

N.B. With old specimens of nicotine, which have become resinous, these crystals are obtained, if at all, with difficulty.

(iv) **Paradimethylaminobenzaldehyde Test.**—Dissolve a pinch of this reagent in a drop of conc. HCl on a watch glass and add from the side a drop of the aqueous solution of nicotine, a rose-red colour develops immediately at the surface of contact, then changes to violet red and subsequently the entire liquid becomes violet red. The red colour gradually intensifies and persists for several hours.

Coniine and pyridine do not give this reaction while aniline yields a red colour and a precipitate of red needles.

(v) **Schindelmeiser's Test.**—To the residue add a drop of 30% formaldehyde solution (free from formic acid), and then a drop of conc. H_2SO_4 or HNO_3 , the mixture becomes rose-red. If the nicotine and formalin be allowed to stand for several hours, a solid compound is formed, which gives a more pronounced rose-red colour on the addition of HNO_3 . Formalin must not be in excess, otherwise the solution becomes green after some time.

This test is not given by coniine, pyridine, aniline or piperidine.

(vi) **Biological Test.**—If 1 c.c. of a dilute aqueous solution of nicotine (1:1000) is injected into the dorsal lymph sac of a frog, it assumes in 5-10 minutes the following position: The forelegs are pressed backward against the sides of the body, the hind legs are drawn up with the thighs fixed at right angles to the body and the feet resting upon the back. The respiration is first accelerated and then becomes slow. Slight muscular spasms in the hind legs may occur. With larger doses, a severe clonic spasm of the body takes place and is followed by fixation of the legs as described above and finally by general muscular relaxation.

Quantitative Determination of Nicotine

Weigh out the minced tissue or measure the stomach-contents and take in a distilling flask, add sufficient water to render it gruel-like in consistency and make it strongly alkaline with caustic soda. Distil in steam and collect the distillate in a flask containing 15 to 20 c.c. of 10 per cent HCl and continue the distillation till no opalescence is produced with the last few drops when tested with silicotungstic acid. Take an aliquot portion of the distillate, add some 10 per cent HCl (about 4 c.c. for each 100 c.c. of the liquid taken) and then add a slight excess of a 10 per cent silicotungstic acid solution. Mix and allow to stand overnight or, if necessary, for 24 hours. Filter the crystalline precipitate, wash thoroughly with water acidulated with HCl until the filtrate becomes free from silicotungstic acid when tested with a drop of the original distillate containing nicotine. Dry and incinerate the precipitate in a platinum crucible. The weight of the incinerated residue minus the weight of the ash of the filter paper gives the amount of silicotungstic anhydride ($12 \text{ WoO}_2 \cdot \text{SiO}_2$). The amount of nicotine in the aliquot portion will be obtained by multiplying the weight of the anhydride by the factor 0.114.

III. NON-VOLATILE ALKALOIDS

Opium Alkaloids

The history of opium is very interesting. It is believed that the poppy (*Papaver somniferum* L.) from which opium is obtained, was first cultivated in Asia Minor for its seeds (for oil) and for the medicinal properties of its fruits (poppy capsules). The Greeks first extracted opium (Gk. *opion*) from the capsules and discovered its poisonous property (Nicander, 185-135 B.C., and Pliny, 70 A.D.). The Arabs further studied its medicinal value and helped to propagate this knowledge to Persia, India and China. The Sanskrit name of opium (*Ahifen*) and the Chinese name (*Ya-pien*) have been derived from its Persian name (*Afy-un*). It was not known to the ancient Egyptians nor is there any reference to *ahifen* in ancient Sanskrit literature.

Opium

It is the air-dried milky exudation or latex obtained by incising the unripe capsules (see Fig. 48) of *Papa-*

ver somniferum L. which is cultivated mostly in Asia Minor, Persia, India and China and to a smaller extent in Greece,

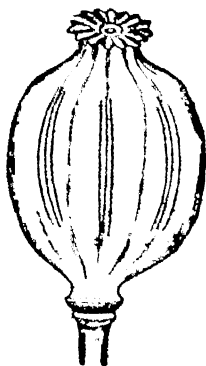


Fig. 48

Belgium, etc., in Europe. The Indian opium was formerly known as Patna opium on account of the fact that the major portion of it used to be manufactured in the Government factory at Patna. The cultivation of *Papaver somniferum* and the manufacture of opium by the Government of India have been considerably curtailed in recent times in accordance with the decision of the League of Nations, and the annual output from the Government Opium Factory at Ghazipur near Benares has been reduced to about 1,000,000 lbs. per annum from about 7,000,000 lbs. 50 years ago. The

Benares opium, as it is called now, is a mixture of opium obtained from Central India and from the districts of Benares, Ghazipur, Lucknow, Azamgarh, etc., in the U.P. Their morphine contents vary from 7 to 14 per cent., but the mixture is standardized in the Government Opium Factory to contain about 10 per cent of morphine. The total alkaloids of this opium usually go up to 40 per cent in which codeine is present to the extent of 1.8 and narcotine 6.4 per cent (Rakshit). This Indian opium differs from opium from other countries by having porphyrroxine, an alkaloid, the composition of which has been worked out by Rakshit and found to be $C_{19}H_{23}NO_4$.

The alkaloids of opium do not exist, in common with the alkaloids of other plants, as free bases but in combination with acids, the most important of which is meconic acid, the other acids being the acetic, lactic, and sulphuric acids. The presence of morphine along with meconic acid in any material in a suspected case of poisoning is, therefore, an indication of opium poisoning.

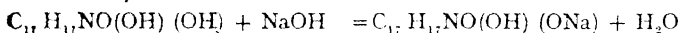
Opium poisoning, mostly suicidal and rarely homicidal, is fairly common in the Punjab and in Bengal. About 35

per cent of cases of fatal poisoning investigated in the Chemical Examiner's Department in Bengal in 1939 were due to opium. In the Punjab, the percentage frequently exceeds 40; it was 42 per cent in 1939.

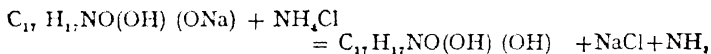
1. Morphine, $C_{17}H_{19}NO_3$, $C_{17}H_{17}NO$ (OH) (OH)

Morphine is very easily oxidized by $KMnO_4$ and other mild oxidizing agents to various derivatives which do not respond to most of the tests for morphine. In opium poisoning, the usual treatment of washing the stomach with $KMnO_4$ solution converts morphine into non-toxic pseudomorphine or oxydimorphine ($2C_{17}H_{19}NO_3 + O = C_{34}H_{36}N_2O_6 + H_2O$). The solubility of morphine in various solvents varies very much. For example, 1 gram of morphine requires for solution 3340 c.c. of water, 6250 c.c. of ether, 1220 c.c. of chloroform, 210 c.c. of alcohol, 125 c.c. of amyl alcohol, and 100 c.c. of lime-water at $25^\circ C$, 1075 c.c. of boiling water, 98 c.c. of boiling alcohol and 50 c.c. of hot amyl alcohol. It is insoluble in benzene.

Morphine is precipitated from its acid solution by ammonia and alkali hydroxides and also by carbonates and bicarbonates, but the precipitate dissolves readily in excess on $NaOH$, KOH or $Ca(OH)_2$ to form soluble morphinates, but not by ammonia and alkali carbonates:



It is, however, reprecipitated from alkaline solutions by ammonium salts:



Extraction of Morphine and other Components of Opium.—As *morphine* and *meconic acid* are two important constituents of opium and as both of them have got very reliable chemical tests, the presence of these two constituents is a positive evidence of opium poisoning and attempts should be made to isolate them as completely as possible and establish their identity in the extracted material. *Porphyroxine*, which is also a characteristic component of Indian opium and which may be extracted along with morphine, affords an additional evidence for the presence of opium.

As morphine is excreted mostly by the kidneys, the urine forms an important material for its extraction. The best materials for extraction of meconic acid are, however, the stomach and its contents although it is found in the liver and other organs.

Procedure.—Take about 200 grams of the finely minced viscera or the stomach contents and submit them to the Stas-Otto method of extraction as described on page 427, and proceed up to the stage (c). The acid aqueous solution contains meconic acid and the alkaloids. Take a portion of this acid solution (the remaining portion being set apart for alkaloids) and treat it with a slight excess of 10 per cent basic lead acetate solution—a precipitate of lead meconate is formed. Heat on a water bath for 5 minutes and allow to settle. Filter. Wash the precipitate on the filter with warm distilled water. Suspend the washed precipitate in about 10 c.c. of water and saturate the suspension with H_2S , lead sulphide is precipitated and free meconic acid is liberated. Remove the lead sulphide by filtration. Concentrate the filtrate to a small bulk so that all traces of H_2S disappear and filter again. Test the filtrate for meconic acid (see p. 489).

Take the remaining portion of the acid solution and render it alkaline with ammonia and then extract with ether instead of chloroform as described under (d) on p. 428; the ether extract will contain the alkaloid porphyroxine $C_{19}H_{23}NO_4$. Evaporate the extract to dryness and test for porphyroxine (p. 489).

The alkaline solution is now ready for extraction of morphine either by amyl alcohol or by chloroform-ether mixture (3:1) as in (e). Extract 3 or 4 times. Evaporate the combined extracts to dryness and purify the residue (particularly of the amyl alcohol extract) by the following method:

Dissolve the residue in hot amyl alcohol, mix the solution with hot water acidulated with sulphuric acid and shake the mixture for a few minutes. The acidulated water takes up morphine while the colouring matter and other impurities are retained by amyl alcohol. Separate the acid aqueous

solution and repeat the process 2 or 3 times. Render the combined acid extracts alkaline with ammonia and extract the alkaline solution with warm chloroform-ether mixture (3:1) several times. Evaporate the combined extracts to dryness. The morphine thus obtained is in a much purer state and is quite suitable for confirmatory tests or for the determination of its quantity.

Tests for Morphine

(i) *Marquis' Test*.—Add a small drop of this reagent (with the point of a glass rod) to the dry residue in a porcelain capsule, a beautiful purple colour is produced which changes to violet and finally to blue.

Marquis' reagent is prepared by adding 3 drops of formalin (40 per cent formaldehyde solution) to 3 c.c. of conc. sulphuric acid.

Heroin gives similar colour reactions with this reagent while codeine gives an initial blue colour and not the purple.

(ii) *Mecke's Test*.—Add a drop of Mecke's reagent to the residue in the same way as in Marquis' test. It gives a blue colour changing to bluish green and olive green.

Mecke's reagent is prepared by dissolving 1 g. of selenious acid in 200 g. of pure conc. sulphuric acid.

Both codeine and heroin also give similar colour changes.

(iii) *Ferric Chloride Test*.—Dissolve the residue in very dilute HCl and evaporate this solution to dryness on the water bath. Dissolve this residue in a few drops of distilled water and add a drop or two of dilute neutral ferric chloride solution—a blue colour develops at once.

This test may be simplified by adding a drop of dilute ferric chloride solution to the dry residue on a porcelain capsule, when a blue colour appears.

The phenolic OH group present in morphine accounts for this reaction. This test is given by oxydimorphine but not by codeine and heroin which are, thus distinguished from morphine. As a specific test for morphine, it is the least delicate of the tests employed for its detection.

(iv) *Iodic Acid Test*.—Dissolve the residue in a few drops of dilute sulphuric acid and add to it a few drops of pure iodic acid (HIO_3) or KIO_3 solution (free from KI) and some chloroform and shake. HIO_3 is reduced and free iodine is liberated which imparts a reddish violet colour to chloroform. This test shows the reducing action of morphine. Other alkaloids such as hydrastine and some ptomaines possessing such reducing action also give this test. But codeine and heroine do not respond to this test.

(v) *Husemann's Test*.—To the solid residue add 2 or 3 drops of conc. sulphuric acid and heat on the water bath for 30 minutes or over a small flame for a few minutes until white fumes appear, a reddish or reddish brown or black colour appears. Cool and add a drop or two of conc. HNO_3 or a crystal of KNO_3 —a reddish violet colour appears which immediately changes to blood-red and then to reddish yellow and finally fades away.

This test depends upon the conversion of morphine into apomorphine and as such it is given by apomorphine. It is also given by codeine and some derivatives of morphine such as oxydimorphine, heroine, dionine, etc.

(vi) *Potassium Ferricyanide Test*.—Dissolve the residue in a few drops of a very dilute solution of HCl ($\text{N}/100$) and evaporate to dryness and dissolve it again in a few drops of water. Add to it a drop or two of a dilute solution of $\text{K}_3\text{Fe}(\text{CN})_6$ and the same amount of a dilute solution of FeCl_3 —a deep blue (Prussian blue) colour develops at once.

In this reaction, morphine reduces $\text{K}_3\text{Fe}(\text{CN})_6$ to $\text{K}_4\text{Fe}(\text{CN})_6$ which produces Prussian blue with FeCl_3 , morphine being oxidized to dioxymorphine. Codeine and heroine do not give this test.

(vii) *Oliver's Test*.—Dissolve the residue in a few drops of dilute HCl , add a few drops of hydrogen peroxide and introduce a small piece of clean copper wire into the solution. Render the solution alkaline with ammonia and when the resulting effervescence ceases, the solution is found to assume a pink or wine-red colour. If the quantity of morphine is very small, the solution becomes blue due to the copper. Add a little KCN solution which will

destroy the blue colour and restore the pink colour due to morphine.

Codeine and heroine do not give this test.

(viii) *Froehde's Molybdic Test*.—Add to the residue one or two drops of *Froehde's reagent* (prepared by dissolving 0.1 g. of sodium or ammonium molybdate in 10 c.c. of conc. H_2SO_4 , heating gently if necessary; the solution should be colourless), a violet colour is produced which changes to blue, green and finally to pink or rose-red. The colours disappear on dilution with water. The reagent should be freshly prepared.

Heroine gives similar colour reactions but codeine does not.

Tests for Porphyroxine

(i) With Mayer's and other alkaloidal reagents it gives a positive test. (ii) To the residue obtained by evaporating the alkaline ether extract, add a few drops of dilute HCl and warm—a beautiful pink or rose-red colour develops.

Test for Meconic Acid

To the filtrate obtained after separation of lead sulphide and expulsion of H_2S , add a few drops of dilute neutral ferric chloride solution—a blood red colour develops which is not discharged by boiling nor by adding a slight excess of HCl (distinction from acetates and formates), nor by mercuric chloride solution (distinction from thiocyanates). The colour is, however, discharged by the addition of stannous chloride but returns on adding nitrous acid.

Quantitative Determination of Morphine

Morphine is easily oxidized and this is the reason why in many known cases of acute poisoning "it has not been detected by competent chemists in viscera even when the examination was started soon after death. But why such a state of things obtains in one case and not in another is beyond our knowledge." In any case the possibility of loss of morphine specially in the decomposed body is very great and the amount obtained by usual methods of quantitative determination gives only the approximate figure (Webster).

Weigh out about 200 g. of the minced material and submit it to the Stas-Otto method of extraction. Evaporate to dryness the

purified chloroform-ether or amyl alcohol extract. Purify the residue as described before. Dissolve the purified residue in a known quantity of N/10 sulphuric acid and titrate with N/20 or N/50 NaOH solution using methyl red as indicator. Each c.c. of N/10 H_2SO_4 combined with the morphine represents 0.02852 g. of anhydrous morphine or approximately 0.2852 g. of opium. From this result the amount of morphine or opium as grams per kilo or grains per pound may be calculated.

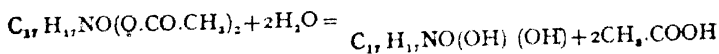
HEROINE (HEROIN) AND CODEINE

Codeine is the natural alkaloid of opium while heroine or diacetyl-morphine is the synthetic derivative of morphine obtained by its acetylation, the H atoms of both the OH groups being replaced by acetyl radical.

Fatal cases of poisoning by codeine or heroine are comparatively rare in this country. Non-fatal accidental cases are occasionally met with.

2. Heroine, Heroin. $C_{17}H_{17}NO(O.O.C.CH_3)_2$

It is readily hydrolyzed by heating with water or with dilute acids but specially with NaOH solution to morphine and acetic acid:



This deacetylation takes place mostly in the system and not in the stomach and as such in fatal cases of poisoning a portion of heroine may be recovered from the tissues as morphine during extraction by the Stas-Otto method. From the stomach contents it may be recovered practically fully as heroine. To prevent its hydrolysis by alkali, the material for extraction by the Stas-Otto process should be made only faintly alkaline with sodium carbonate and extracted at once with the suitable solvent. Heating above $50^\circ C$ is to be avoided during the whole process of extraction.

Heroine is very readily soluble in chloroform. One gram is dissolved by 1.4 c.c. of $CHCl_3$, 31 c.c. of alcohol, and 100 c.c. of ether at $25^\circ C$. It is also readily soluble in benzene (cf. morphine).

Extraction of Heroine—Extract by the Stas-Otto process using a suitable alkali for precipitation and chloroform as the solvent. The different stages of extraction are the same as in morphine (see).

Tests for Heroine

The difficulties in obtaining the typical colour reactions as stated under codeine are also met with in detecting heroine. There is another difficulty and that is the readiness with which it is hydrolyzed to morphine and acetic acid when heated with water, acids, or caustic alkalies. For this reason heroine responds to all the tests for morphine in which conc. sulphuric acid is used, e.g., in Marquis, Mecke, Husemann, Froehde and other tests.

The Ferric Chloride, Iodic Acid, and other tests which distinguish codeine from morphine are, therefore, applicable to heroine. The following additional tests may also be depended upon:

(1) *Zernik's Test*.—To the residue add a few drops of conc. HNO_3 (sp. gr. 1.4 = 60 per cent) and rub with a glass rod—a yellow colour is produced which soon changes on warming to a greenish blue colour and gradually fades again to yellow.

(2) Heat the residue with sulphuric acid and add a little chloral hydrate solution—a brownish red colour appears.

(3) Heat heroine (about 0.1 g.) with 1 c.c. of alcohol and 1 c.c. of conc. sulphuric acid—the odour of ethyl acetate is recognized. This test is only successful if sufficient amount of heroine is available, for instance, in identifying suspected substances found on the person of the deceased, or in connection with examination of drugs under the Dangerous Drugs Act, or for identification of the contents of unlabelled poison bottles.

3. Codeine, $\text{C}_{18}\text{H}_{21}\text{NO}_3$, $\text{C}_{17}\text{H}_{17}\text{NO}(\text{OH}) (\text{O}.\text{CH}_3)$

Chemically it is methyl morphine, the hydrogen atom of the phenolic OH group of morphine being replaced by

the methyl radical. Codeine is very readily soluble in chloroform. One gram of codeine is dissolved by 0.5 c.c. of chloroform, 2 c.c. of alcohol, 13 c.c. of benzene, and 18 c.c. of ether at 25°C. It is also soluble in amyl alcohol. It differs from other alkaloids by its high solubility in water (1 in 120), and in ammonia. In this respect it differs from morphine which dissolves in caustic alkalies but not in ammonia. On account of this property it is not precipitated by ammonia but only by caustic alkalies and alkaline carbonates.

Extraction of Codeine—Same as in Heroin.

Tests for Codeine

The tests for codeine, heroine and morphine as described under morphine show the typical colour changes only where pure alkaloids are tested but on account of the presence of impurities invariably found with these alkaloids when they are extracted in minute quantities from post-mortem materials and when further purification is not feasible on account of the total loss of alkaloids, the characteristic colour reactions as described in text books are seldom obtained. For this reason as many tests as possible are to be applied to the residue. The tests which distinguish morphine from codeine or heroine by slight differences in colour reactions should not be depended upon in expressing a definite opinion one way or the other.

The Ferric Chloride, Iodic Acid, Potassium Ferricyanide, Froehde's and Oliver's tests, which are all negative with codeine, are useful to distinguish it from morphine. Since morphine is soluble in excess of caustic alkalies and codeine is not, the latter can be completely extracted by ether from an aqueous alkaline solution. This property lends an additional support to the confirmatory tests for codeine.

4. Strychnine, $C_{21}N_2O_2H_{22}$

Strychnine is the most toxic alkaloid of *Nux Vomica* (*Strychnos nux vomica* L.) which grows wild in most parts of India and particularly in Orissa. Strychnine and another alkaloid brucine occur principally in the seeds of *Strychnos nux vomica* and *Strychnos ignatii* and in the root bark

of *Strychnos tieuté* the deadly upas tree known as African arrow poison. Fig. 49 shows the flower, fruit and the seed of *Strychnos nux vomica*. The bark of *Strychnos toxifera* is the common source of South American arrow poison curare (curarine) which produces motor paralysis. The *nux vomica* seeds are known as *Kuchila* which is used in the indigenous systems of medicine as also for criminal purposes. The other alkaloid brucine is not of any medicolegal importance.



Fig. 49

Strychnine is intensely bitter, much more than any other known substance. It is precipitated from its solution by ammonia and other alkali hydroxides and carbonates and is not soluble in excess of these reagents. It is readily soluble in chloroform. One gram is dissolved by 5 c.c. of CHCl_3 , 136 c.c. of alcohol or 180 c.c. of benzene at 25°C , or by 34 c.c. of boiling alcohol. It is slightly soluble in ether.

Extraction of Strychnine.—As strychnine is eliminated mostly through the urine, the latter, if available, should be examined separately and particularly for the determination of its quantity.

The material should be submitted to the Stas-Otto process, using ammonia for alkalization and chloroform for extraction. In cases of poisoning by *nux vomica* (*Kuchila*) both strychnine and brucine are extracted together and as the presence of brucine interferes with the tests for strychnine it is essential to separate strychnine from brucine.

Separation of Strychnine from Brucine.—Dissolve the chloroform residue containing strychnine and brucine in about 2 c.c. of dilute sulphuric acid, add 2 drops of conc. HNO_3 and allow to stand for 30-60 minutes at $15^\circ\text{-}20^\circ\text{C}$. Alkalinize strongly with NaOH solution and extract several times with chloroform. Wash and evaporate the combined chloroform extracts to dryness—the residue is free from brucine and is thus fit for the usual tests for strychnine.

Tests for Strychnine

(i) **Dichromate Test.**—To the brucine-free residue on a white porcelain surface, add a drop of pure conc. H_2SO_4 —no colouration is noticed. Take a crystal of $\text{K}_2\text{Cr}_2\text{O}_7$ and draw it by means of a glass rod through the sulphuric acid—a play of colours will be observed, first a *momentary blue* changing to a beautiful violet colour which gradually changes to reddish purple, red or orange and finally to yellow. In the presence of very small qualities of strychnine, the play of colours is very rapid while with larger quantities the violet tint persists for some time before it changes to other colours.

In the presence of impurities, the drop of conc. H_2SO_4 when added to the residue may cause slight darkening which, however, does not interfere with the test excepting the detection of the initial transient blue colour which is, however, not an important phase of this colour reaction.

Instead of using $\text{K}_2\text{Cr}_2\text{O}_7$, other oxidizing substances, such as manganese dioxide, cerium oxide, KMnO_4 , vanadic acid, etc., may be used successfully. *Neither nitric acid nor any nitrate should, however, be used as they interfere with or even prevent this test. For this reason strychnine nitrate may not give this test.*

(ii) **Mandelin's Test.**—To the brucine-free residue add a drop of Mandelin's reagent (one per cent solution of ammonium vanadate in conc. H_2SO_4)—a deep violet-blue or deep purple colour appears, which is more permanent than with dichromate test, and finally changes to yellow on long standing.

(iii) **Malaquin's Test.**—Dissolve the residue in a few drop of dilute HCl , add 2 c.c. of conc. HCl and about one

gram of pure granulated zinc. Allow to stand for 2-3 minutes, heat quickly to boiling and cool. Transfer to a small test tube and add gently down the side of the test tube some conc. H_2SO_4 —a rose-red ring will develop at the junction of the two liquids and subsequently the whole mixture becomes rose-red. This colour is destroyed by adding a solution of potassium thiocyanate or ammonia or NaHSO_4 in excess.

(iv) *Physiological Tests for Strychnine.*—(a) The bitterness of strychnine is more intense and more persistent than that of any other alkaloid or any other known substance. If a drop of a dilute solution of a strychnine salt is applied to the tongue, it appears to spread out rapidly all over the buccal cavity. The bitterness of a solution of a concentration of 1 in 600,000 may be perceived by one who is particularly sensitive to bitter taste. *The absence of bitter taste of the residue is a definite proof that strychnine is not present.*

(b) Dissolve the residue in a few drops of dilute (N/100) HCl. Evaporate this solution to dryness and dissolve the residue in 5 or 6 drops of water. Inject this solution with a hypodermic syringe into the dorsal or ventral lymph sac of a healthy frog and place it under a bell jar. Toxic symptoms which develop in about 5—30 minutes, depending upon the quantity of strychnine injected, are as follows:—The hind legs become extended and stiff with tetanic spasms or convulsions of the whole body at frequent intervals. During the remission, the hind legs remain flexed. Later on the convulsions become more frequent and the hind-legs are kept extended and stiff and the front legs are crossed. Slight tapping on the table or gently touching the animal with cotton wool produces convulsions. Finally, the convulsions are replaced by gentle tremors which persist for some time and then the animal dies. It is stated that 0.02 to 0.05 mg. of strychnine is the minimal dose to produce convulsions in a medium-sized frog, but it appears to be much less in our experience.

Quantitative Determination of Strychnine

Dissolve the residue (after destruction of brucine) in a known quantity of N/10 H_2SO_4 solution. Titrate the excess of acid with

N/50 NaOH solution, using methyl red as an indicator. Each c.c. of N/10 H_2SO_4 combining with strychnine corresponds to 0.03343 g. of strychnine.

5. Brucine, $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_4$

Poisoning by brucine is not known and can not be traced in the literature. In experimental animals it produces convulsions but the dose necessary to give rise to such condition is 40—50 times more than that for strychnine. On the other hand its curare-like (paralysis) action is much stronger.

As Kuchila (nux-vomica seed) has frequently been the cause of poisoning in this country, it is essential to detect the presence of brucine in order to prove a case of nux-vomica poisoning in contradistinction to strychnine alone.

Tests for Brucine

(i) *Nitric Acid Test*.—To the chloroform residue (see p. 494) containing both brucine and strychnine, add a drop of conc. HNO_3 , a blood-red colour appears which changes to reddish yellow and finally to pure yellow. Add a few drops of dilute stannous chloride solution to the reddish yellow or yellow solution, an intense purple colour develops which is destroyed by adding a drop of conc. HNO_3 . To carry out this test successfully, it is desirable to add the smallest quantity of HNO_3 at the initial stage of the test.

(ii) *Blyth's Test*.—Dissolve the residue in alcohol and add a few drops of methyl iodide—in a few minutes crystals of methyl brucine iodide appear as rosettes. An alcoholic solution of strychnine gives no such crystals.

6. Atropine, $\text{C}_{17}\text{H}_{23}\text{NO}_3$

The alkaloid atropine is obtained along with hyoscyamine and a few other alkaloids from several plants belonging to the Natural Order Solanaceæ. The important members of this group are *Atropa belladonna*, *Datura stramonium*, *Datura fastuosa*, and *Hyoscyamus niger*. Of these, *Datura fastuosa* is of special toxicological value on account of its extensive criminal use in this country. It grows all over India and its seeds are fairly rich in these

alkaloids. The powdered seeds are used by professional poisoners for purposes of robbery and theft usually in rail-

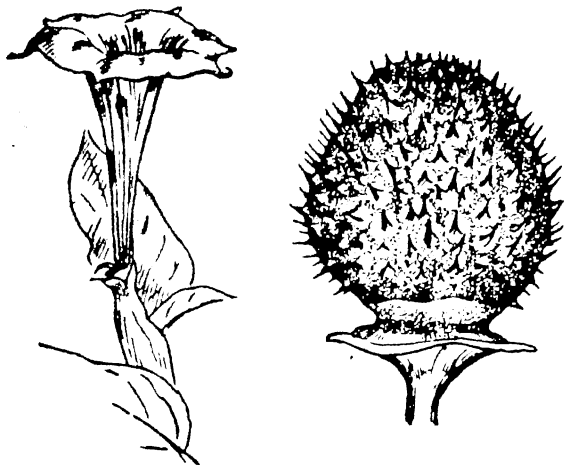
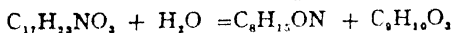


Fig. 50

way trains and places of pilgrimage, and very seldom for any homicidal purpose. Fig. 50 shows the flower and fruit of *D. fastuosa* var. *alba*—the common white flowered *datura*.

It has been suggested that the alkaloid atropine does not occur in more than traces in solanaceous plants and that during the process of extraction the l-hyoscyamine originally present in the plant is converted into dl-hyoscyamine or atropine (Henry). The conversion of hyoscyamine (lævo-rotatory) into atropine (racemic) may be effected in the laboratory simply by heating it to its melting point or by treatment with alkalis. Atropine is, therefore, stereoisomeric with hyoscyamine.

Atropine is a complex ester of tropic acid and is readily hydrolyzed by warming with alkalis, dilute acids, or by boiling with water, into tropine, $C_8H_{15}ON$, a basic secondary alcohol, and tropic acid, $C_9H_9O_3$, a homologue of mandelic acid, as shown in the following equation:



Tropine is optically inactive but tropic acid is optically active and is capable of existing in *d*- and *l*-forms. In atropine it is present in its *dl*- or racemic form. As both the components are optically inactive atropine and its salts are also optically inactive. A *d*-hyoscyamine has been prepared artificially from tropine and *d*-tropic acid. This artificial alkaloid is toxicologically less potent than its isomers and their positions may be expressed thus: *l*-hyoscyamine > *dl*-hyoscyamine (atropine) > *d*-hyoscyamine. Atropine, therefore, occupies an intermediate position as far as its toxicity is concerned.

Atropine is readily soluble in chloroform, benzene, amyl alcohol and other organic solvents, e.g., one gram is dissolved by 27 c.c. of glycerol, 25 c.c. of ether, 2 c.c. of alcohol and 1 c.c. of chloroform at 25°C. It is sparingly soluble in petroleum ether. Of all alkaloidal reagents, Mayer's reagent is the most delicate and reliable for detecting atropine in small quantities in biological materials, e.g., in blood serum.

Extraction of Atropine.—The extraction of this alkaloid from the urine, blood, viscera and other materials is made by the Stas-Otto process, special care being taken to prevent its hydrolysis. The extraction should be carried out at a relatively low temperature using the minimum quantities of tartaric acid for acidification and avoiding NaOH or other strong alkalis for alkalization—sodium carbonate being most suitable. Chloroform should be the solvent of choice.

Tests for Atropine

(i) **Vitali's Test.**—To the extracted residue in a porcelain dish add 1 to 2 drops of fuming or conc. HNO₃, and evaporate to dryness on the water bath. Allow it to cool and add a few drops of freshly prepared alcoholic potash—a fine violet colour is produced which soon changes to red and then disappears. On adding a few drops more of alcoholic potash, the colour reappears.

This reaction is given by all solanaceous alkaloids, with the exception of homatropine—an artificial alkaloid prepared by the condensation of tropine and mandelic acid.

(ii) **Gerrard's Test.**—To the purified residue, add drop by drop, 1 to 2 c.c. of a 2 per cent solution of mercuric

chloride in 50 per cent alcohol, avoiding excess of the reagent—a red colour appears at once.

Hyoscyamine gives a yellow colour which becomes red on warming. No yellow or red colour is obtained with hyoscine (scopolamine).

(iii) *Physiological Tests*.—Dissolve the purified residue in a few drops of $N/100$ H_2SO_4 and instil 1 or 2 drops of this solution into the conjunctival sac of a cat. In a few minutes the pupil begins to dilate. The dilatation is best observed by comparing this pupil with that of the other eye. The observation should be made in an open place with plenty of light so that the normal dilatation which occurs in a shady or a dark place may not be mistaken for dilatation by atropine and the animal should not get frightened in any way, as fright also dilates pupils. One drop of atropine solution (1 : 130,000) will cause distinct dilatation of the pupil in a cat (Autenrieth). The dilatation by atropine persists for several hours (cf. cocaine, p. 506).

It is stated that certain cadaveric alkaloids from highly decomposed viscera may produce similar mydriasis and may be mistaken for atropine but the fact that they do not respond to the chemical tests described above differentiates them from atropine (see page 432).

Quantitative Determination of Atropine

Dissolve the purified residue in neutral alcohol and add to it a known amount of $N/50$ H_2SO_4 and dilute with a little distilled water. Titrate with $N/50$ $NaOH$ solution using methyl red as indicator. Each c.c. of $N/50$ H_2SO_4 combined with the alkaloid represents 0.005786 g. of atropine.

7. Aconitines—The Aconite Alkaloids

The aconitines form an important group of highly toxic alkaloids found mostly in the roots of different species of *Aconitum*. Only a few of them grow in the temperate and sub-alpine regions of the Himalayas and the roots obtained from this source are usually known as 'Nepal aconite roots'.

The well known alkaloid aconitine, the empirical formula of which is now accepted as $C_{34}H_{47}O_{11}N$, is found in *Aconitum napellus* which does not grow in this country. Only three aconitines, viz., pseudaconitine $C_{36}H_{51}O_{12}N$.

indaconitine $C_{34}H_{47}O_{10}N$ and bikhaconitine $C_{34}H_{47}O_{11}N$ are found in four species of *Aconitum* growing in India. The names of these species are :

- | | |
|-------------------------------------|---|
| 1. <i>Aconitum Balfourii</i> Stapf. | } Originally included under the name <i>Aconitum ferox</i> . They are the sources of pseudoaconitine. |
| 2. <i>A. deinorrhizum</i> Stapf. | |
| 3. <i>A. chasmanthum</i> Stapf. | This is the principal source of Bish or Bikh of the Calcutta market. It contains indaconitine. |
| 4. <i>A. spicatum</i> Stapf. | Also known as <i>A. ferox</i> . This is the source of bikhaconitine. |

Of these three alkaloids, pseudoaconitine is the most toxic. Indaconitine and bikhaconitine are less toxic than the former, but more toxic than aconitine (Henry). The facts that we do not know exactly the variety of aconite available in the market and thus accessible to the public and the source from which it is obtained and that all the aconitines produce the same kind of tingling sensation in the mouth and show the same toxic signs and symptoms, preclude the toxicologist from going into details about the actual identity of the alkaloid. This is the reason why the toxicologists in India state in their reports that 'aconite' has or has not been detected in the material examined by them and do not refer to any of the aconitines.

Aconite root is used in the powdered form as a homicidal poison in Bengal and other parts of India. The powdered root of Indian aconites is slightly sweetish in taste and hence it is called *Mitha Zahar* (sweet poison). Aconite is therefore most suitable for administration with food without arousing any suspicion on the part of the victim. It is also used as an arrow poison by the Nagas and other hill tribes in Assam and Burma frontier regions. It appears from the literature that the species of aconitum (*A. napellus*) available in the West is not sweet but bitter and its bitter taste is attributed to its amorphous alkaloids and not to aconitine.

Aconitine and its closely related alkaloids mentioned above are weakly alkaline to litmus, readily soluble in chloroform and benzene (1 part in 7), less readily in alcohol (1 in 28) and in ether (1 in 65) and sparingly in petroleum ether. The bases are dextrorotatory but their salts are levorotatory. They are very unstable alkaloids, easily undergoing hydrolysis in the presence of acids and alkalies and even by boiling with water. This property presents considerable difficulty in isolating them from tissues and other materials, the final residue obtained after completing the processes of extraction being the hydrolysis products of the alkaloid.

Hydrolysis Products of the Aconitines.—Aconitine gives acetic acid and benzoyleaconine when hydrolyzed by boiling with dilute acids, and gives acetic acid, benzoic acid and aconine, $C_{25}H_{41}O_9N$, if hydrolyzed by alkalies.

Pseudaconitine yields acetic acid and veratroyl pseudaconine if its neutral salt is boiled with water: acetic acid, veratric acid and pseudaconine, $C_{25}H_{41}O_8N$, if hydrolyzed with alcoholic sodium hydroxide (even without heating).

Indaconitine yields acetic acid, benzoic acid and pseudaconine, if hydrolyzed with alcoholic soda (without heating).

Bikhaconitine yields acetic acid, veratric acid and bikhaconine $C_{25}H_{41}O_7N$, if hydrolyzed with alcoholic soda (without heating).

Extraction of the Aconitines —As aconitines are eliminated through the urine and saliva, these fluids are valuable for isolation of the alkaloids. The materials are to be submitted to the Stas-Otto method of extraction. The preliminary digestion with alcohol should be conducted at room temperature for sometime and finally at about $60^{\circ}C$ for an hour or so. The acidification should be done carefully with tartaric acid, the reaction being only slightly acid. The evaporation of the filtrate should be carried out at room temperature under a vacuum and alkalization must be done with sodium bicarbonate. Both ether and chloroform should be used as solvents for final extraction (see p. 429). The use of ice box for keeping the extracted materials during the intervening stages of extraction is helpful.

Tests for Aconite Alkaloids

The tests given below can only be successfully carried out if pure alkaloid is obtained for the test. But in actual practice it is not possible to purify the extracted alkaloid to perfection and the chemical tests are therefore not dependable. The analyst has, therefore, to fall back upon the physiological test only which we consider infallible, if the test is carried out by an experienced toxicologist.

(i) *Gold Chloride Test*.—Dissolve the extracted residue in 2 or 3 drops of dilute ($N/100$) HCl . Add a few drops of gold chloride solution (5 per cent)—an amorphous precipitate is formed which shows golden yellow needles or rectangular prisms if crystallized from alcohol.

(ii) *Vitali's Test*.—See atropine (p. 498.) Pseudocomitine gives this test.

(iii) *Alvarez Reaction*.—Add to the purified residue in a porcelain dish 5 to 10 drops of pure bromine and evaporate on the water bath. Add 1 to 2 c.c. of conc. HNO_3 and evaporate to dryness, adding again a few drops of bromine if the nitric acid loses its colour. To the yellow oxidation product add 0.5 to 1 c.c. of a saturated alcoholic solution of $NaOH$ and again evaporate to dryness. A red or brown residue is obtained. Allow to cool and add 5 to 6 drops of a 10 per cent $CuSO_4$ solution—a green colour develops.

(iv) *Palet's Reaction*.—Add to the purified residue a few drops of a mixture consisting of 25 g. of syrupy phosphoric acid (85 per cent) and 1 g. of sodium molybdate. Heat over a small flame until vapours appear—a violet colour develops. Veratrine also gives this test.

(v) *Physiological Tests*.—(a) Dissolve the residue in a few drops of very dilute HCl and apply a drop of this solution to the tip of the tongue by gently rubbing with the finger. In 5 to 15 minutes, a tingling sensation develops which is not confined to the tip but gradually spreads out (cf. oleander). If the amount of the alkaloid is not very small, a very uncomfortable sensation of dryness of the throat along with a choking sensation is felt. It persists for several hours (cf. oleander). This test is very characteristic and may be safely relied upon. The chemical tests described

above are only dependable if confirmed by the physiological tests.

(b) If a few drops of the above solution are injected into the dorsal or ventral lymph sac of a frog (not water frog), the hind limbs become extended and flaccid, respiratory difficulties appear with copious secretion of a lymph-like exudation all over the body. Finally, the respiration fails with retraction of the chest wall on either side and the frog dies in about half an hour or even earlier.

8. Cocaine. $C_{17}H_{21}NO_4$

Fatal cases of poisoning by cocaine are not infrequent in this country, particularly among the cocaine addicts who take cocaine hydrochloride to enjoy the 'feeling of exhilaration and sense of happiness and well being which transport at once to a longed-for elysium'.

Cocaine is the methyl benzoyl ester of a basic substance ecgonine $C_9H_{15}NO_3$. When heated with mineral acids, it is hydrolyzed into ecgonine, benzoic acid and methyl alcohol. If it is boiled with water, it splits up into methyl alcohol and benzoyl ecgonine which in turn can be hydrolyzed by acids or alkalis into ecgonine and benzoic acid.

It is very readily soluble in $CHCl_3$, benzene and other solvents. One gram is dissolved by 0.7 c.c. of $CHCl_3$, 3.5 c.c. of ether, 6.5 c.c. of alcohol and 12 c.c. of olive oil at $25^\circ C$. It is also soluble in benzene but is insoluble in glycerol. Its solution is alkaline to litmus and methyl orange but not to phenolphthalein.

Extraction of Cocaine.—As cocaine is readily hydrolyzable, it is rapidly decomposed in the tissues. The toxicologists are of opinion that chemical examination almost always gives negative result unless cocaine be taken in large quantities. As a rule, little can be isolated from the viscera after a few hours. It has been suggested that ecgonine, the basic hydrolysis product of cocaine, should be detected in the urine and tissues, but so far no satisfactory method has been worked out for its detection.

The extraction of cocaine is made, as usual, by the Stas-Otto method. It should be carried out at a low temperature with the minimum amount of tartaric acid for acidification, and for alkalization sodium carbonate or preferably bi-

carbonate should be used. The solvent for extraction should be either chloroform or benzene.

Tests for Cocaine

Many tests have been recommended for the detection of cocaine, but the amount usually obtained, if at all, by extraction from viscera is hardly sufficient for such tests.

The following tests are, however, useful for detecting cocaine in suspected substances found on the person or in the rooms of the deceased or in adulterated samples seized from cocaine smugglers, or in connection with cases under the Dangerous Drugs Act:

(i) *Alum-Permanganate Test*.—Dissolve the residue or a small quantity of the suspected substance in a few drops of



Fig. 51

very dilute (N/100) HCl and evaporate to dryness. Dissolve this residue in a few drops of a saturated solution of alum. Take a drop of this solution on a microscope slide and add to it a drop of a saturated solution of

KMnO_4 and mix them together by gently rubbing on the slide with a glass rod for about a minute or two—characteristic violet-red or pink rhombic (almost rectangular) plates of cocaine permanganate are found under the microscope (see Fig 51).

If the drops of cocaine and KMnO_4 solutions are mixed gently on the slide and the slide is left uncovered and allowed to evaporate almost to dryness, larger crystals are found (Bagchi et al).

Antipyrine interferes with this test and should be removed. Novocaine does not give this test.

(ii) *Gold Chloride Test*.—Dissolve the residue or the suspected substance in a few drops of dilute (N/100) HCl. Take a drop of this cocaine hydrochloride solution on a microscope slide and add to it a small drop of gold chloride solution (5 per cent)—at once an amorphous precipitate is formed which rapidly becomes crystalline. Examine under the microscope—the crystals resemble fern-fronds and are very characteristic. It is a delicate test and detects cocaine even in a concentration of 1 in 20,000. (See Fig. 52).

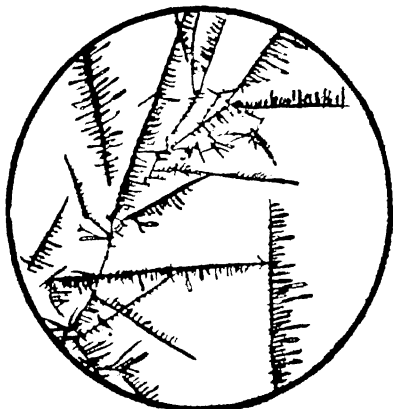


Fig. 52

Novocaine also gives similar crystalline precipitate, but the precipitate is soluble in dilute HCl while the cocaine gold chloride crystals are insoluble in HCl.

(iii) *Iodic Acid Test*.—To the substance add a few drops of conc. H_2SO_4 and a small particle of pure KIO_3 or HIO_3 . No colour develops in the cold. Now heat the mixture on the porcelain dish over a small flame until the vapours of H_2SO_4 come off or even a little longer, when 'appear in succession and together brown, olive green, blue and violet shades of colour which issue from the particle of iodate and immediately disappear. Finally, vapours of iodine are also given off.' This test detects 0.05 mg. of cocaine. Other alkaloids also give intense colourations with H_2SO_4 and KIO_3 but usually in the cold.

Ecgonine, under these conditions of the test, gives cherry to purple red colour which finally changes to dirty violet.

(iv) *Chromic Acid Test*.—Dissolve the substance in a few drops of very dilute HCl and evaporate to dryness.

Dissolve this dry residue in a few drops of water and to this aqueous solution of cocaine hydrochloride add a drop or two of 5 per cent solution of $K_2Cr_2O_7$ —a yellow precipitate is produced which redissolves on shaking the mixture. Now add to this clear solution about 1 c.c. of conc. HCl—an orange coloured precipitate of fine needles is obtained. This reaction is shown in a concentration of 1 : 1000.

(v) *Physiological Tests*.—(a) Convert the residue into cocaine hydrochloride as described under the chromic acid test and apply a drop of the solution to the tongue, repeating the contact on the same spot—a sense of numbness and a characteristic insensibility to touch will be perceived which last for a few minutes only.

(b) Instil a drop of the cocaine hydrochloride solution into the eye of a cat—a dilatation of the pupil with anæsthetic and blanching effects on the conjunctiva is observed. The dilatation is however not so well marked and persistent as noted with atropine (see p. 499).

Cocaine Substitutes—Novocaine or Procaine

Of the common cocaine substitutes used in medicine, novocaine or procaine $C_{11}H_{20}N_2O_2$ is toxicologically important on account of its extensive use as an adulterant of cocaine which is smuggled to the cocaine addicts, and cases of fatal poisoning by taking large doses of adulterated cocaine containing mainly novocaine are occasionally met with. As novocaine is not included in the schedules under the Dangerous Drugs Act, it is readily accessible to the public. Since the beginning of the war with Japan it has not been available to the addicts.

Extraction of Procaine.—The extraction of procaine from the tissues and its detection present the same difficulties as in the case of cocaine. If the examination is made soon after death, it may be possible to extract this basic substance from the organs and particularly from the liver, but such attempts have usually been unsuccessful. The methods of extraction are the same as in cocaine.

Tests for Procaine in the presence of Cocaine

(i) *Diazo Test*.—Dissolve the residue in a few drops of very dilute HCl and evaporate to dryness. Add about 2 c.c.

of water, 2 drops of conc. HCl and a 10 per cent solution of sodium nitrite and mix them carefully with a glass rod. To the mixture add drop by drop about 3 c.c. of β -naphthol reagent (prepared by dissolving 0.4 g. of β -naphthol in 10 c.c. of 10 per cent NaOH solution)—a scarlet red precipitate is produced.

(ii) *Aldehyde Test*.—Take a drop of procaine solution or a minute crystal of it on a microscope slide and add to it a drop of *p*-dimethylamino-benzaldehyde reagent (prepared by dissolving 1 g. of *p*-dimethyl amino-benzaldehyde in 95 c.c. of absolute alcohol and 20 c.c. of conc. HCl)—a yellow or a greenish yellow colour develops at once (Chakravarty and Roy).

Cocaine and other alkaloids do not give this test. Orthoform and anæsthesine give this reaction but they differ from procaine by their insolubility in water.

Quantitative Determination of Cocaine in the Presence of Novocaine and other Adulterants of Cocaine (Method of Bagchi *et al*)

Weigh out about 0.5 g. of the sample and dissolve in about 10 c.c. of 20 per cent HCl in a small beaker. Add 2.5 c.c. of an one per cent gold chloride solution drop by drop until the precipitation of cocaine gold chloride is complete; stirring all the time with a glass rod. The precipitate is at first amorphous, but soon becomes crystalline. Allow two hours to settle completely and then filter through a small quantitative filter paper. Wash the residue in the filter with 15 c.c. of water (5 portions of 3 c.c. each) till the filtrate is free from novocaine as indicated by the test described above. Perforate the filter and wash the residue with 20 c.c. of water into a separating funnel. Render strongly alkaline with 10 per cent solution of ammonia which decomposes the double salt cocaine-gold chloride and liberates the cocaine base. Extract cocaine five times with anæsthetic ether (B.P.). Wash the combined ether extracts with about 5 c.c. of water. Evaporate the washed ether extract in a platinum dish on a steam bath. Dry it in an air oven at 70°C to constant weight. This weight gives the amount of cocaine base present in the original solution. Multiplied by the factor 1.12 the weight of cocaine hydrochloride will be obtained (for further details see *Ind. Med. Gaz.*, 1939).

Instead of weighing the evaporated ether extracts, it may be dissolved in a known amount of $N/50$ H_2SO_4 and titrated with $N/50$ NaOH with methyl red as an indicator. One c.c. of $N/50$ H_2SO_4 is equivalent to 0.00679 g. of cocaine hydrochloride.

CHAPTER XXXV

Non-alkaloidal and Indigenous Poisons: Barbiturates. Salicylic acid and its Derivatives. Acetanilide. Phenacetin. Sulphonamides. Plumbago. Oleanders Indian Hemp. Jequirity. Croton. Madar. Cleistanthus collinus. Marking Nut.

I. NON-ALKALOIDAL ORGANIC POISONS

1. Barbiturates

A large number of derivatives of barbituric acid or malonyl urea (see p. 259) are used in medicine as hypnotic drugs and some of them are very popular and taken indiscriminately without consulting a physician and thus become the sources of accidental poisoning by overdosage. Cases of suicide by these drugs are also met with. The following barbiturates are of toxicological importance in this country:

- (i) Veronal, Barbital, Barbitone, Diethyl barbituric acid, $\text{CO}(\text{HN}.\text{CO})_2.\text{C}(\text{C}_2\text{H}_5)_2$.
- (ii) Medinal, Sodium barbitone, Soluble barbital, $\text{CO}(\text{HN}.\text{CO}) (\text{NaN}.\text{CO}).\text{C}(\text{C}_2\text{H}_5)_2$.
- (iii) Luminal, Gardenal, Phenobarbital, Phenylethyl-barbituric acid, $\text{CO}(\text{HN}.\text{CO})_2.\text{C}(\text{C}_2\text{H}_5) (\text{C}_6\text{H}_5)$.

The *solubility* of these barbiturates is as follows:

Veronal is soluble in 130 parts of water, 75 parts of chloroform, 35 parts of ether, 14 parts of alcohol at 25°C and 13 parts of boiling water. It is also soluble in acetone and ethyl acetate. Its solution is acid to litmus.

Medinal is soluble in 5 parts of water at 25°C and in 2.5 parts of boiling water. It is slightly soluble in alcohol but insoluble in ether. Its aqueous solution is alkaline to litmus.

Luminal is soluble in about 1,000 parts of water, 700 parts of benzene, 40 parts of chloroform, 13 parts of ether, and 8 parts of alcohol at 25°C. It is soluble in alkali hydroxides and carbonates.

Of these barbiturates, veronal is readily eliminated through the kidneys. As much as 90 per cent may be recovered from the urine which is, therefore, the most important material for toxicological investigation. Luminal, on the other hand, is not excreted in the urine. Brain and spleen as also the other organs retain a considerable amount of this drug. The post mortem materials for toxicological analysis should, therefore, include the liver, kidney, brain, spleen, and the stomach and its contents.

Extraction of Veronal and other Barbiturates.—

The barbiturates are recovered from the acid ether extract of the Stas-Otto method of extraction (see p. 428, para c.). In a suspected case of barbiturate poisoning, the initial acidification should be done with dilute H_2SO_4 and the extraction of the acid aqueous filtrate with ether. Then evaporate the ether extract to dryness, purify the crystalline residue by taking up with a little hot water and decolourize by boiling with animal charcoal. Filter hot and evaporate the filtrate to dryness. The crystalline residue will be quite pure for determination of the melting point.

As animal charcoal causes considerable loss of the material by adsorption, the following alternative method of purification, especially useful for quantitative determination, may be adopted: Take up the crystalline residue with a little hot water acidified with dilute H_2SO_4 , heat to boiling and add drop by drop 5 per cent solution of KMnO_4 just to give a slight pink colour to the solution. Filter and decolourize the filtrate with a few drops of hydrogen peroxide. Extract with ether and evaporate to dryness, the residue will be quite pure.

For extraction of veronal from the urine, concentrate it to syrupy consistency and extract with 95 per cent alcohol acidified with dilute H_2SO_4 on a steam bath for about an hour. Filter and evaporate the filtrate almost to dryness.

Digest with hot water and filter. Extract with ether and proceed further with the stages described above.

Tests for Veronal and Medinal

The tests for veronal are not at all satisfactory—most of them being of negative character as against the positive colour reactions given by other barbiturates. If the extracted drug is sufficiently pure and available in large quantity, the tests are likely to be useful but these possibilities are usually remote in toxicological investigations. The following tests are useful in identifying veronal and medinal:

(i) *Millon's Reagent*.—Dissolve the residue in a small amount of warm water and add a few drops of Millon's reagent—a gelatinous white precipitate is formed which is insoluble in excess of the reagent.

(ii) *Cobalt nitrate Test*.—To an alcoholic solution of the residue add a drop or two of ammonia to render it alkaline and then add a few drops of alcoholic solution of cobalt nitrate—a violet colour is produced.

N.B.—These two tests are general tests for all derivatives of barbituric acid and not specific tests for veronal or medinal.

(iii) *Melting Point*.—The m.p. of dry purified veronal is 191°C (corrected) but in actual toxicological analysis this figure is seldom obtained possibly due to the presence of impurities. Autenrieth gives the m.p. of pure veronal as 187° — 188°C , Webster as 187° — 190° , and so on. It is, therefore, desirable to determine the m.p. of the purified residue after carefully mixing it with the same quantity of pure veronal. If the residue in question is veronal, the mixture will melt at the same temperature as either substance alone; if the two are not identical, the mixture will give a lower melting point.

N.B. If the residue containing veronal is heated in a dry test tube, veronal sublimes in well formed crystals which may be used for determining the m.p.

(iv) To the residue add a little solid NaOH and fuse, ammonia is evolved. Dissolve the fused mass in water; the solution gives a blue colour with FeSO_4 solution and a purple colour with CuSO_4 solution.

(v) Dissolve the crystalline residue in an excess of Na_2CO_3 solution and boil—veronal is decomposed with evolution of ammonia.

(vi) *Van Ittalie and van der Veen's Test.*—Dissolve the residue in water rendered faintly alkaline with ammonia and add to this an equal volume of ammoniacal solution of AgNO_3 (a 5 per cent AgNO_3 solution to which just sufficient ammonia is added to redissolve the precipitate of silver oxide first formed)—a white precipitate rapidly forms which redissolves in hot water and recrystallizes on cooling in colourless, birefringent crystals.

Tests for Luminal

(i) *Melting Point.*—The m.p. of pure luminal is stated to be 172° — 174°C but usually it is about 170°C .

(ii) To the residue in a porcelain basin add one or two drops of the *Marquis reagent* (see morphine) and allow to stand for a minute or two and then warm on the water bath—olive green and violet patches appear and then the whole mass becomes reddish purple.

(iii) *Ramweiz's Test.*—To the residue add about 5 times the amount of KNO_3 and about 2 c.c. of conc. H_2SO_4 and boil for 10 minutes. Pour the yellow solution into 10 c.c. of cold water—a heavy crystalline precipitate appears. Now add a large excess of ammonia to this mixture—the precipitate dissolves giving a yellow solution.

N.B. This test is given by all barbiturates having the aromatic ring.

(iv) *Ehkert's Test.*—To the residue, add about 1 c.c. of formalin and 4 c.c. of conc. H_2SO_4 and heat the mixture in a water bath, the mixture acquires a rose-red to wine-red colour after a few minutes (yellow colour with veronal).

Quantitative Determination

The residue purified as described under extraction is dissolved in ether and transferred to a weighed beaker. Evaporate the solvent. dry the residue at about 100°C to constant weight which gives the

amount of the barbiturate present in the weighed quantity of the material

2. Salicylic Acid and its Derivatives

Poisoning by salicylic acid and its derivative aspirin or acetyl salicylic acid is occasionally met with. They are mostly accidental and in some cases suicidal. Salicylic acid circulates in the blood as sodium salicylate, and is found in all the organs and secretions of the body. *It is not found in the faeces.*

Salicylic Acid, $C_6H_4(OH).COOH$

It is sparingly soluble in cold water but readily in boiling water. One gram of salicylic acid is dissolved by 460 c.c. of water, 135 c.c. of benzene, 42 c.c. of chloroform, 3 c.c. of ether or acetone, and 2.7 c.c. of alcohol at 25°C.

Extraction of Salicylic Acid.—As salicylic acid is volatile in steam, it is easily extracted from the viscera, urine and other materials by steam distillation after acidification with dilute H_2SO_4 . The distillate contains salicylic acid and some salicyluric acid if the poisoning is due to a salicylate or salicylic acid, and it contains salicylic acid along with some phenol if salol (phenyl salicylate) is responsible for poisoning.

Render the distillate alkaline with Na_2CO_3 and extract several times with ether—salicylic acid remains in the alkaline solution as sodium salicylate while phenols, etc., are extracted by ether. Separate the alkaline solution and acidify with H_2SO_4 —salicylic acid is liberated. Extract with ether and evaporate the ether extract to dryness—the crystalline residue is pure salicylic acid.

The acid ether extract of the Stas-Otto process also contains salicylic acid with or without phenol according to the nature of the poisoning.

Tests for Salicylic Acid

(i) **Ferric Chloride Test.**—Dissolve the residue in hot water or in dilute alcohol, add a few drops of dilute $FeCl_3$ solution—a violet colour develops which is not discharged by alcohol (distinction from phenol, see p. 447).

(ii) *Bromine Water Test*.—Add bromine water drop by drop—a white precipitate of tribromosalicylic acid $C_6HBr_3(OH)COOH$ is formed (see p. 448).

(ii) *Methyl Salicylate Test*.—To the residue add one c.c. of methyl alcohol and 0.5 c.c. of conc. H_2SO_4 and heat till the sweet smell of methyl salicylate is recognized.

(iv) *Jorissens' Test*.—Dissolve the residue in 10 c.c. of water by heat and transfer to a test tube and allow to cool. Add 4 or 5 drops of 10 per cent KNO_3 solution, 4 or 5 drops of 50 per cent acetic and one drop of 10 per cent copper sulphate solution. Mix and boil for about a minute or two—a red colour develops.

Quantitative Determination of Salicylic Acid

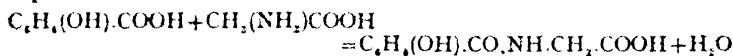
The extraction of salicylic acid from viscera by steam distillation as described on p. 512 is not suitable for quantitative determination as complete extraction is not likely by this method. The following method is useful: Weigh out the finely minced tissue and mix with water acidified with H_2SO_4 to the consistency of a thin gruel. Heat on the boiling water bath for about two hours. Filter hot and wash with hot water. Render the filtrate and the washings alkaline with Na_2CO_3 solution and evaporate to dryness. Dissolve the residue in dilute H_2SO_4 . Extract this acid solution repeatedly with ether. Transfer the combined ether extracts to a separating funnel and shake vigorously with about 15 c.c. of dilute sodium carbonate solution, when salicylic acid will pass into the aqueous solution as sodium salicylate while fat and other impurities (phenols, &c.) will remain in the ether. Separate the alkaline aqueous extract and repeat this process of extraction for 2 or 3 times more. Combine the alkaline aqueous extracts, acidify with dilute H_2SO_4 (1:3) and again extract with ether 2 or 3 times. Wash the combined ether extracts with water until the washings are free from acid. Evaporate the washed ether extract to dryness. Dissolve the crystalline residue of salicylic acid in a small quantity of hot water, dilute with water and after cooling make up to a definite volume. Take an aliquot portion of this solution dilute with water and add to it a few drops of 0.5 per cent solution of $FeCl_3$, until the maximum colour is developed (avoiding an excess of the reagent). Match this colour in a colorimeter or in Nessler tubes against the colour obtained with standard solution of salicylic acid (containing 1 mg. of salicylic acid in 100 c.c. of water similarly treated with $FeCl_3$ and calculate the amount of salicylic acid accordingly.

In the case of urine or other liquid materials, acidify with dilute H_2SO_4 and extract with ether repeatedly. Treat the ether extracts with dilute Na_2CO_3 solution and proceed according to the method described above.

Aspirin $C_6H_4(O.CO.CH_3).COOH$

It is readily hydrolyzed into salicylic and acetic acids by boiling with water or by alkali hydroxides and carbonates in the cold in which aspirin is readily soluble. One gram of aspirin is dissolved by 300 c.c. of water, 17 c.c. of chloroform, 12 c.c. of ether and 5 c.c. of alcohol at $25^{\circ}C$. It melts at about $132^{\circ}C$.

Aspirin and salicylates are eliminated in the urine in the forms of salicylic and salicyluric acids, the latter being a conjugate of salicylic acid with glycine which is supplied by the system. The conjugation is indicated by the following equation:



Both these acids give positive ferric chloride test. The salicyluric acid may, however, be hydrolyzed by refluxing with strong HCl.

Extraction of Aspirin—As aspirin is very easily hydrolyzed even by hot water, the tissues or the urine or any material suspected to contain aspirin should be extracted with warm absolute alcohol. Filter the alcoholic solution and dehydrate the filtrate with anhydrous Na_2SO_4 and then evaporate to dryness. Extract the residue with ether. Evaporate the combined ether extracts to dryness. The crystalline residue contains aspirin with a small proportion of salicylic acid which is usually formed in the system by hydrolysis of aspirin and also to a certain extent during the process of extraction. Test this residue for aspirin.

Tests for Aspirin

(i) *Ferric Chloride Test*.—Dissolve the residue in cold water, filter to separate free salicylic acid if present, and to the filtrate add a few drops of dilute $FeCl_3$ solution—a yellow brown colour develops if no free salicylic acid is present. But as a trace of salicylic acid is likely to be present in the solution, $FeCl_3$ would give a slight purple tint instead of yellow brown. Boil the aqueous solution for a few minutes, cool and then add $FeCl_3$ solution—an intense violet colour develops.

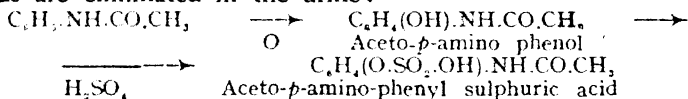
(ii) Dissolve the residue in about a c.c. of dilute NaOH solution, boil, cool in ice and add a drop or two of dilute H_2SO_4 —a white precipitate of salicylic acid is formed and the odour of acetic acid is recognized. Filter to remove the salicylic acid and test the filtrate for acetic acid (see p. 142).

3. Acetanilide, Antifebrin

Poisoning from acetanilide $C_6H_5.NH.CO.CH_3$ is mostly accidental due to overdosage of the drug and cases of attempted suicide are also reported. Fatal cases are rare.

Acetanilide is readily soluble in alcohol, ether, chloroform and acetone and in boiling water. It is soluble in 190 parts of water at $25^\circ C$. It melts at $114^\circ C$.

Acetanilide as such is not eliminated in the urine unless large doses are taken. Most of it is oxidized in the human system to aceto-*p*-amino phenol which undergoes conjugation with sulphuric and glycuronic acids and these conjugated acids are eliminated in the urine:



Extraction of Acetanilide—Extract the finely minced tissue or other material with rectified spirit for several hours. Filter, evaporate the alcohol and take up the residue with water acidified with sulphuric acid. Extract the acid solution with ether or chloroform. Evaporate the solvent to dryness and test the residue for acetanilide.

As the urine is not likely to contain acetanilide, a large quantity of it should be boiled with 10 c.c. of conc. HCl under a reflux condenser for an hour or two which results in the breaking down of conjugated bodies and liberation of *p*-amino phenol. The mixture is cooled and rendered alkaline with excess of Na_2CO_3 and extracted several times with ether. On evaporating the ether extracts, *p*-amino phenol is obtained as a brownish oily residue. To an aqueous solution of this residue, the indo-phenol test may be applied.

Tests for Acetanilide

(i) *Indophenol Test*.—This test is given by compounds containing a free primary aromatic amino group. As aceta-

nilide possesses no free amino group it must first be hydrolyzed by HCl as described above.

Take a portion of the residue and boil with 4 c.c. of conc. HCl in a test tube until it is reduced to 1 c.c. Cool and add 2-4 c.c. of saturated aqueous phenol solution. Add drop by drop with good shaking a freshly prepared solution of calcium hypochlorite, a dirty red colour is produced, which deepens if the mixture is shaken for a few minutes. Then carefully layer some ammonia on this mixture—the upper layer becomes indigo blue whereas the lower layer retains the red colour (see p. 477). Phenacetin also gives this test.

(ii) *Phenyl-isocyanide Test*.—Boil a small portion of the residue with a few c.c. of alcoholic or aqueous NaOH solution—the odour of aniline will be perceptible. Then proceed with the test as described on p. 283.

(iii) *Ethyl Acetate Test*.—Take a large portion of the residue in a test tube, add to it a few drops of conc. H_2SO_4 and a little alcohol and heat for a few minutes in a boiling water bath—the smell of ethyl acetate will appear on carefully adding a few drops of water.

This test is due to hydrolysis of acetanilide and liberation of acetic acid which forms the ethyl acetate. This test alone has got no value unless it is combined with the phenyl-isocyanide test.

Quantitative Determination

Transfer the residue extracted from a known quantity of the material (viscera, vomit, remnants of substances suspected to contain acetanilide, etc.) to a flask, add 20 c.c. of 20 per cent HCl and boil for 15 minutes under a reflux. Make up to 100 c.c. with water. To 25 c.c. of this solution (filtered if necessary) in a stoppered bottle, add 5 c.c. of conc. HCl and 15 c.c. (or more) of N/10 bromine (bromate-bromide) solution and allow the mixture to stand for 15 minutes in the dark. Add an excess of KI and titrate the liberated iodine with N/10 $Na_2S_2O_3$ solution. Since 1 c.c. of N/10 bromine = 0.002252 gm. of acetanilide, the amount present in the material taken for examination is calculated accordingly.

4. Phenacetin, Acetophenetidin, Aceto-*p*-phenetidine, *p*-Ethoxy acetanilide

As in the case of acetanilide, the poisoning from phenacetin $C_6H_5(O.C_2H_5).NH.CO.CH_3$ is mostly accidental.

Phenacetin differs from acetanilide by having an ethoxyl group in the para position. It is sparingly soluble in cold water but fairly readily in alcohol, chloroform and ether. It is soluble in 2.8 parts of boiling alcohol. It is hydrolyzed by heating with NaOH solution or conc. HCl into *p*-phenetidine and acetic acid and not into aniline and acetic acid as in the case of acetanilide. In moderate doses, phenacetin undergoes hydrolytic changes in the human body with formation of *p*-phenetidine and *p*-amino phenol. These products are excreted with the urine either by themselves or as conjugates of sulphuric and glycuronic acids. As *p*-amino phenol is responsible for indophenol reaction, phenacetin also gives the indophenol test.

Phenacetin-urine is frequently yellow coloured and reduces Fehling's solution on account of having an excess of glycuronic acid in combination with the hydrolysis products mentioned above. *This urine differs from glycosuric urine in not fermenting with yeast.*

Extraction of Phenacetin.—It is extracted from tissues and urine in the same way as described under acetanilide.

Tests for Phenacetin

(i) *Indophenol Reaction.*—Positive (see acetanilide).

(ii) *Phenyl Isocyanide Test.*—Negative (see pp. 67, 283 and 516).

(iii) *Oxidation Test.*—Boil the residue with 1-3 c.c. of conc. HCl for three minutes or more. Dilute with water to about 10 c.c. cool and filter. Add to the filtrate a few drops of 3 per cent chromic acid or 8 per cent $K_2Cr_2O_7$ solution or some strong chlorine water, a violet colour changing rapidly to ruby red develops. Acetanilide gives a yellow colour which slowly changes to green.

(iv) *Periodide Test.*—To the residue transferred into a test tube, add a drop of glacial acetic acid, 0.5 c.c. of water, and 1 c.c. of N/10 iodine solution; warm the mixture to about 40°C and add a drop of strong HCl, a crystalline precipitate of periodide of phenacetin $(C_2H_5O.C_6H_4NH.CO.CH_3)_2HI.I_4$

separates in the form of reddish brown leaflets or needle-shaped crystals. Shaking and warming hasten the reaction.

The formation of crystalline periodide is the basis of a gravimetric method of determination of phenacetin.

Quantitative Determination

Transfer the extracted residue from a known amount of the material to a 50 c.c. conical flask, add 2 c.c. of glacial acetic acid, heat gently over a wire gauze to dissolve it, and dilute with 40 c.c. of warm water (70°C). Transfer the solution to a 100 c.c. volumetric flask containing 15 c.c. of N/5 iodine solution warmed to 40°C, wash the flask twice with 10 c.c. of warm water (40°C) and add the washings to the solution. Mix, add 3 c.c. of strong HCl and shake until the crystalline periodide $[(C_8H_7O_2C_6H_4NH.CO.CH_3)_2HI.I_2]$ begins to form and then set aside to cool. Gentle agitation of the flask in a warm water bath (temp, not exceeding 40°C) hastens the formation of crystals. Dilute nearly to 100 c.c., mix, and allow to stand overnight. Make up to 100 c.c., mix and allow to stand 30 minutes. Filter through a dry filter paper and titrate 50 c.c. of the filtrate with N/10 $Na_2S_2O_3$ solution.

1 c.c. of N/5 iodine solution = 0.01791 gm. of phenacetin.

5. Sulphonamides or Sulphanilamides

The sulphonamide or sulphanilamide group of drugs, the chemical nature of which has been discussed before (see p. 294), are now used extensively in medicine and cases of acute poisoning by these drugs have lately been reported. In most cases, overdosage or the susceptibility of the individual to this class of drugs, is the cause of poisoning and even death. It has also been noticed in a few cases that imperfect preparation of the drug resulting in its decomposition during storage has been the cause of poisoning.

The following sulphonamides have been known to produce acute symptoms of poisoning: (1) Prontosil album or sulphanilamide, (2) Prontosil rubrum, (3) Soluseptasine, and (4) M. & B. 693 or Dagenan.

Extraction from Viscera, Stomach-contents, etc.—

Take a known quantity of the finely minced material and to it add an excess of acetone (about twice the weight of the material), warm on a water bath for about an hour with thorough shaking at frequent intervals, cork the flask and leave it overnight at room temperature. Filter through a

fluted filter paper, evaporate the filtrate to a syrupy consistency and again extract with acetone in the same way. Filter, wash with acetone several times and evaporate the combined filtrates to dryness. Dissolve the residue in about 20 c.c. of water by heat and filter hot. If there is much colouring matter, decolourize with animal charcoal, cool and *saturate the filtrate with NaCl*. Transfer to a separating funnel, add about 10 c.c. of acetone, shake vigorously and allow to separate into two layers (a portion of NaCl being precipitated). Transfer the upper layer (acetone containing some water) to an evaporating basin and repeat the acetone extraction several times. Evaporate the combined acetone extracts on the steam bath to dryness; the residue consists of the sulphonamide and some NaCl. Dissolve the residue in water and apply the specific tests described below. An aliquot portion may be used for quantitative determination.

Tests for Sulphonamides

(i) To a small fragment of the residue or to a few drops of its solution, add a drop or two of the *p*-dimethylaminobenzaldehyde reagent (prepared by dissolving 3 g. of *p*-dimethylaminobenzaldehyde in 100 c.c. of water containing 7 c.c. of conc. H_2SO_4)—at once a yellow colour or orange precipitate appears. This test is given by all aryl-amino compounds.

(ii) Dissolve a portion of the residue in 2 c.c. of warm dilute HCl (10%). Cool in ice and add 2 c.c. of a 1 per cent solution of sodium nitrite; add 2 c.c. of water and 1 c.c. of 5% β -naphthol solution (prepared by dissolving 5 g. of pure β -naphthol in 40 c.c. of 20% NaOH solution and then making up to 100 c.c.), an orange colour or precipitate is produced.

(iii) Sulphanilamide when heated in a dry test tube, an intense violet colour is produced and on further heating odours of NH_3 and aniline are emitted.

Quantitative Determination in Urine and Blood (Werner's Method)

Urine.—Dilute the urine (100-200 times) with distilled water so that the diluted solution may contain 0.5 to 1.5 mgm. of the sulphonamide per 100 c.c. To 9 c.c. of the diluted urine add 1 c.c. of the

p-dimethylaminobenzaldehyde reagent—the yellow colour develops immediately. Match this colour against similar colour obtained with standard sulphanilamide solution treated exactly in the same way. For comparison in the usual types of colorimeter, a 1 mg. standard solution set up at 20 mm. will serve the purpose nicely.

Blood.—Add 1 c.c. of oxalated blood drop by drop from a pipette, to 4 c.c. of a 5 per cent solution of trichloroacetic acid. Gently shake the mixture for about a minute and filter through dry filter paper. Take 2 c.c. of the protein-free filtrate, add 1.5 c.c. of N/4 NaOH solution to neutralize the acid and then add 0.5 c.c. of the aldehyde reagent and match the yellow colour in a colorimeter against the standard solution.

Nessler cylinders may also be used in matching the colour and a series of standards corresponding to 0.25 mgm., 0.50 mgm., 0.60 mgm., 0.80 mgm., 1.00 mgm., and 1.50 mgm. of sulphanilamide per 100 c.c. of the solution should be set up for comparison.

Instead of preparing fresh standards every time a determination is carried out, a permanent set of standards with perfectly stable colour may be prepared from 1 per cent solution of pure potassium chromate as given in the following table:

Dilution of one per cent solution of K_2CrO_4	Equivalent con- centration of sulphanilamide (mgm. per 100 c.c.)	Dilution of one per cent solution of K_2CrO_4	Equivalent con- centration of sulphanilamide (mgm. per 100 c.c.)
50 times	.. 0.25	10 times	.. 0.9
25 "	.. 0.50	8.5 "	.. 1.0
20 "	.. 0.60	7.5 "	.. 1.1
15 "	.. 0.70	5.0 "	.. 1.2
12.5 "	.. 0.80	4.5 "	.. 1.3

Acetyl Sulphanilamide and Total Sulphanilamide

It has been shown that in man a portion of sulphanilamide is conjugated with acetic acid and excreted in the urine as acetyl-sulphanilamide which may be determined by the following procedure: Measure out 1 c.c. of the urine in a test tube, add 2 c.c. N HCl and heat in boiling water for about half an hour, when the acetyl derivative is hydrolyzed into acetic acid and free sulphanilamide. Cool the solution, neutralize the acid with 2 c.c. of N NaOH solution and dilute with distilled water to a volume of 10 c.c. Again dilute this solution 1 in 20 or 1 in 40 according to its probable concentration and take 9 c.c. of the dilute solution in a test tube and add 1 c.c. of the aldehyde reagent and match the yellow colour against the standard solution. This result gives the total sulphanilamide, i.e., the free sulphanilamide originally present in the tissue plus the sulphanilamide liberated from its acetyl derivative. The difference between the total and the free gives the amount of acetyl sulphanilamide eliminated by the kidneys.

The acetyl derivative is also found in the blood and can be determined exactly in the same way as described above. In this case 2 c.c. of the blood-filtrate is heated for 30 minutes in boiling water (without adding N HCl), allowed to cool and then neutralized with N/4 NaOH solution before the addition of the aldehyde reagent.

Modification of Werner's Method by Morris

Some of the disadvantages of Werner's method have been got rid of by the following modifications introduced by Morris (Biochem. J. 1941, and Analyst 1942): One c.c. of whole blood is laked by shaking it vigorously with 13 c.c. of water and then allowing it to stand for 3 minutes. The proteins are precipitated by adding 6 c.c. of 20 per cent solution of *p*-toluene sulphonic acid in N/5 HCl and after standing for 5 minutes the precipitate is filtered off. The free sulphanilamide is determined in a 5 c.c. portion of the filtrate by treatment with 1 c.c. of 0.75 M disodium hydrogen citrate solution (prepared by dissolving 39.4 g. of citric acid in 188 c.c. of 2 N sodium hydroxide solution and diluted to 250 c.c.) and 2 c.c. of 2 per cent alcoholic solution of *p*-dimethylamino-benzaldehyde. The yellow colour, which immediately develops its maximum value and is stable for a week, is matched in a colorimeter against the standard.

The total sulphanilamide (free and its acetyl derivative) is determined on another 5 c.c. portion of the filtrate, heating it for an hour in a boiling water bath, the conjugated sulphanilamide being hydrolyzed by *p*-toluene sulphonic acid. After cooling, the solution is diluted to 5 c.c. with water and the colour is developed as before.

II. INDIGENOUS POISONS

1. Plumbago

Two species of plumbago, viz., *Plumbago zeylanica* L. (known as *chitra* or *chita* in Bengal and Northern India, *venchittira* and *tellachitra* in the Madras Presidency) and *Plumbago rosea* L. (*lal-chitra* or *lal-chita*), belonging to the N.O. Plumbaginaceæ, grow wild in Bengal and other parts of India.

The plant is widely used as an abortifacient. The powdered root is taken internally, or made into a paste and applied externally to the os uteri for procuring criminal abortion. The root or stem of the plant is also used as an 'abortion stick' which acts mechanically. Fatal poisoning has been known to occur through overdosage when given by the mouth.

Active Principles of Plumbago.—A golden yellow, crystalline bitter substance, *plumbagin*, is obtained from the

roots of both species of plumbago. It is insoluble in cold water and moderately soluble in hot water but readily soluble in ether, chloroform, alcohol, acetone, etc. On oxidation by neutral or alkaline KMnO_4 in the cold, plumbagin yields benzoic and cinnamic acids whereas by distillation with zinc dust it produces naphthalene and methyl naphthalene. Its formula as suggested by Roy and Dutt is $\text{C}_{11}\text{H}_{15}\text{O}_5$ (*Ind. J. Med. Res.*, Vol. 20, 1933). In small doses it causes contraction of the muscles of the uterus, intestines and heart and in large doses it causes death from respiratory failure. Externally, the extract from the roots acts as a powerful irritant and vesicant. When applied to the skin in the form of a paste, the root produces reddish brown marks simulating bruises caused by injury. This property is taken advantage of by litigant village people to support false charges of assault in village feuds. Solution of plumbagin (1 in 1000) when applied to the skin produces erythema and higher strengths cause painful blisters.

Extraction of Plumbagin—The stomach contents, vomit, etc., usually contain powdered root from which plumbagin is extracted in the following way: Proceed as in the Stas-Otto method of extraction (see para *a*, p. 427). To the residue from the evaporated alcoholic extract, add a small quantity of water, render slightly alkaline with caustic potash solution, and thoroughly mix. Allow to settle for a few minutes, filter, acidify the filtrate with dilute HCl and extract with ether several times. Evaporate the combined ether extracts to dryness. The residue contains plumbagin which may now be submitted to the following tests:

Tests for Plumbagin

(i) To a portion of the residue add a few drops of dilute caustic potash solution—a crimson colour develops. Add a few drops of dilute HCl —the colour changes to yellow, and on standing for some time, a yellow flocculent deposit of plumbagin is formed which is soluble in ether.

(ii) Dissolve the residue in a small quantity of alcohol, add a few drops of a solution of basic lead acetate—a crimson precipitate is formed.

N.B. The extraction of this poison from viscera is not always successful due to decomposition of this substance in the organs. The residue from visceral extracts gives, if at all, the colour reactions only very faintly.

(iii) *Physiological Test*.—A dose of 0.01 mgm. or a higher dose per gramme of body weight when injected into a frog produces drowsiness and ultimately paralysis but no convulsions take place. Higher doses always produce diaphoresis in a frog.

2. The Oleanders

The name oleander has been given to three different species of plants belonging to the N.O. Apocyanaceæ. They are as follows:

1. *Nerium odorum* Sol. (or *N. odoratum* Lam.), the red or white flowered oleander which is grown all over India for its sweet scented flowers. Its roots, barks and leaves are often used in Western and Southern India and also in Bengal as a paste or decoction for suicidal purpose and occasionally as an abortifacient. It is known as *Karabi* in Bengali and *Alari* in Tamil.

A cardiac glucoside, *folinerin*, $C_{29}H_{46}O_8$ has been isolated from its leaves. It is 2-3 times more toxic than digitoxin. It is used for the treatment of myocardial deficiency—gives a more constant and rapid action.

2. *Thevetia neriifolia* Juss. (or *Cerbera thevetia* Linn.), the yellow flowered oleander or yellow oleander (Hindi—*Pila kaner*, Bengali—*Kolkey phul*, and also *Karabi* in East Bengal, and *Thangalari* in Tamil) which grows wild all over India. The kernel of the seed and also the leaves and other parts of the plant are generally used as a suicidal poison, and occasionally as a cattle poison and rarely as a homicidal poison. See Fig. 53.

3. *Cerbera odollam* Gaertn. It is known as *odollam* in Southern India. The kernel of the seed is commonly used as a suicidal poison in the Madras Presidency and in the States of Cochin and Travancore.

Active Principles of the Oleanders

(i) *Nerium odorum*.—A glucoside *nerin* having the formula $C_{35}H_{50}O_{10}$ (m.p. $123^{\circ}C$) isolated and studied by

Naidu and co-workers. It is readily soluble in chloroform, acetone and alcohol, but sparingly soluble in ether, petroleum ether, benzene and water. It is highly toxic and when injected into the dorsal lymph sac of a frog, it produces convulsions followed by paralysis and death. The minimum fatal dose for a frog weighing 10 g. is about 0.02 mgm. It gives an immediate pink colour with conc. H_2SO_4 (Naidu *et al*, *Jour. & Proc. Inst. Chem.*, 1943).

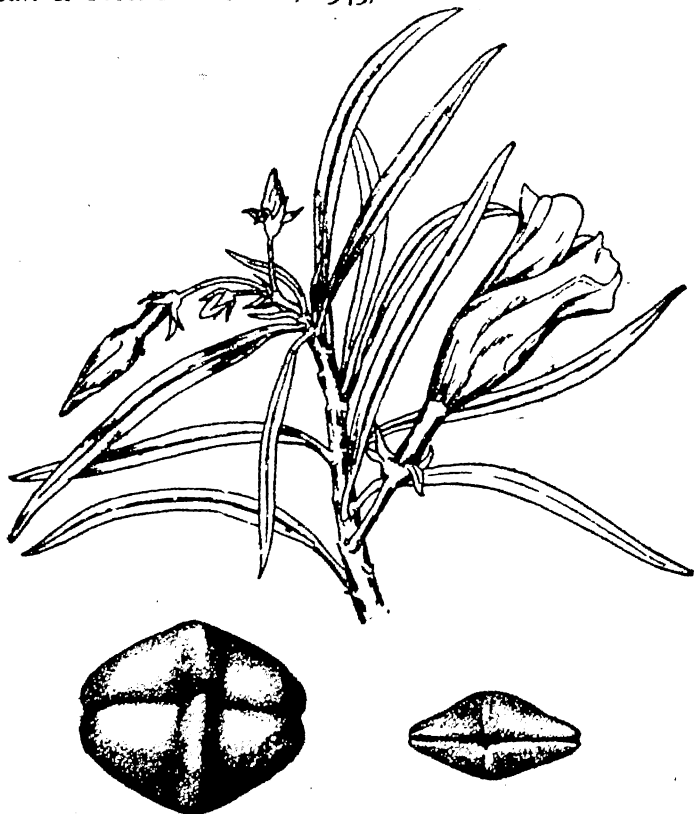


Fig. 53.

(ii) *Thevetia nerifolia*.—(a) *Thevetin*, a glucoside having the formula $\text{C}_{27}\text{H}_{42}\text{O}_{12}$ (m.p. 178°C) suggested by Naidu. It is readily soluble in chloroform and ether but sparingly in water. It is also highly toxic and when injected

into a frog it produces convulsions followed by paralysis and death. The minimum fatal dose is the same as that of *nerin*. (b) *Cerberin*, another glucoside having the formula $C_{20}H_{38}O_{14}$. It is soluble in water and alcohol but insoluble in ether, chloroform and benzene. It is also toxic, the minimum fatal dose being 0.025 mgm. for a frog weighing about 10 g. The paralytic signs are not so well marked as in *nerin* or *thevetin*. *Cerberin* gives a blue or bluish green colour when heated with dilute HCl (Naidu *et al*, *loc. cit.*).

According to Ghatak (Bull. U. P. Academy of Sciences, 1932), the two glucosides present in this plant are *thevetin* $C_{20}H_{30}O_6$ (m.p. 192°C and *thevetoxin* $C_{16}H_{24}O_8$ (m.p. 178°C) Chen and Steldt (Jour. Pharmacol., 1942) also isolated two toxic glucosides, *cerberoside* ($C_{41}H_{70}O_{20}$, m.p. 187° — 188°C) and *cerberin*.

(iii) *Cerbera odollam*.—*Cerberin* $C_{20}H_{38}O_{14}$, the same glucoside as described under *Thevetia nerifolia* (Naidu *et al*).

Extraction of the Oleander Glucosides.—The glucosides of oleander are readily destroyed by hydrolysis with dilute HCl, by the gastro-intestinal secretions, and possibly by the enzymes of the tissues and as such they are rarely detected in viscera. The portions of the crude poison, such as particles of the kernel, root-bark or the leaves which have been left unattacked in the stomach, may be picked up from the stomach contents and vomit and submitted to the Stas-Otto process of extraction, and the acid ether or chloroform extract obtained from stage *c* described on p. 428 may yield the glucosides *nerin* and *thevetin* but no *cerberin* as it is insoluble in ether and chloroform. *Cerberin* may, however, be obtained from the purified acid alcoholic filtrate (in stage *a*) by precipitating it with excess of ether.

Tests for the Oleanders

(i) The preliminary Reinsch test for arsenic which is carried out as a routine measure in all cases of poisoning shows a blue or bluish green colouration of the boiling hydrochloric acid mixture if portions of crude yellow oleander or *odollam* are present in the stomach contents. This is due to the action of hot HCl on *cerberin*.

(ii) To the purified acid ether extract on a porcelain capsule add a drop of conc. H_2SO_4 or H_3PO_4 followed by warming on the water bath—an immediate pink colour appears if *nerin* is present, and a yellowish brown colour slowly changing to pink if *thevetin* is present.

(iii) *Keller's Test*.—Dissolve the ether extract in 1 c.c. of glacial acetic acid containing 5 per cent ferric sulphate and layer this solution over conc. H_2SO_4 containing 0.05 per cent of ferric sulphate, when an immediate crimson in the sulphuric acid layer and a green colour in the acetic acid layer develop if *nerin* is present. If *thevetin* is present, an immediate blue in the acetic acid layer and a mauve colour in the H_2SO_4 layer appear.

N.B. The above two colour tests are only dependable if the residue is pure and in sufficient quantity, the conditions being rarely obtainable in extracts from visceral matters.

(iv) *Physiological Tests*.—(a) Moisten the acid ether extract with a drop of water and apply it to the tip of the tongue by rubbing repeatedly with the finger for a few seconds; it tastes bitter and a characteristic tingling sensation with a feeling of denudation of the epithelium develops in a few minutes. The sensation is quite different from that produced by aconite, and is confined to the spot where it is applied and does not extend to other parts of the tongue as in the case of aconite (see p. 502). This is the most reliable test for *Nerium odorum* and *Thevetia nerifolia*. Minute traces of the glucosides of these oleanders produce the characteristic tingling sensation.

(b) Dissolve the residue in about one c.c. of water and inject the solution into the dorsal lymph sac of a frog—in a few minutes convulsions set in which are followed by paralysis and death. This test requires slightly larger amount of the glucoside than what is required for the tongue test.

3. Indian Hemp. *Cannabis sativa* Linn. (*Cannabis indica* Lam.)

This plant belongs to the N.O. Urticaceæ and grows all over India. It was known in ancient times in India and there is reference to *bhāṅg* (Sanskrit *bhāṅga*) in the Vedic literature. It is cultivated by provincial Governments as their monopoly and yields a substantial excise revenue. In

Bengal it is cultivated in Nowgong in the district of Rajshahi. Elsewhere it is a prohibited plant, though occurring as a roadside weed in many places.

The leaves, flowering tops and the resin obtained from leaves and stems of *C. sativa* are used as intoxicants. The mature leaves, mixed with its fruits, are known as *bhang* or *siddhi* which is taken as a beverage or *sharbat* to produce slight intoxication followed by sleep. The flowering tops of the female plant with its resinous exudation, pressed and matted, are known as *ganja* while the resin exuding from the leaves, young twigs, stems and even from the flowering tops is called *charas* or *hashish* which is a greyish brown pasty substance pressed into cakes. Both *charas* and *ganja* are smoked in small *chilams* either in pure form or mixed with tobacco. In Egypt *hashish* is usually smoked in cocoanut *hookah* known as *goza*, and also eaten as a special preparation containing sugar, butter fat and various flavouring substances. The *charas* available in this country is imported from Chinese Turkistan (Yarkand). It is also prepared in this country from *Cannabis* plants which grow at an altitude of 6 to 8,000 feet in the districts of Garhwal and Kumaon. The *bhang* or *siddhi* is also used in making *majun* or *modak* with sugar, ghee, spices, etc., which the Kavirajes prescribe as an appetizer or aphrodisiac, an overdose of which causing intoxication of a sensuous character.

The cases of poisoning from different Indian Hemp products are usually accidental due to overdosage, although homicidal attempts are occa-

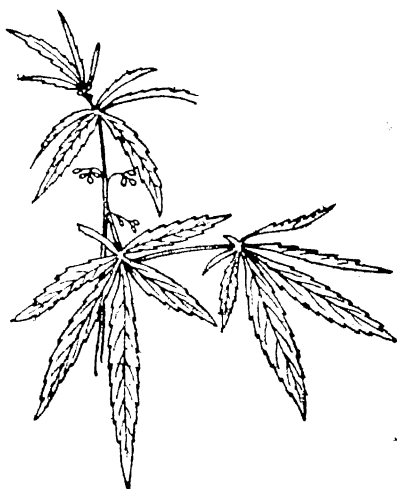


Fig. 54.

sionally made by administering them with food or drink or with tobacco for smoking. They are also criminally used as stupefying drugs for purposes of robbery by the professional poisoners in railway trains (see *datura*). They have lately been used in this country for 'doping' race horses.

Active Principles of *C. sativa*.—Many resinous substances such as cannabinal, cannabinone, etc., believed to be the active principles of *ganja* or *charas* have been described by different workers but until recently no serious efforts appear to have been made to determine their exact chemical composition and to assay their physiological activities. Cannabinal, an oily substance obtained by distilling the resin, was believed to be a pure substance with a definite chemical composition and was held to be responsible for the physiological action of *C. sativa*. It was subsequently found that the cannabinal of one worker was not identical in chemical composition with that of a later worker. Recently Work, Bergel and Todd (*Biochem. Jour.*, Part I, 1939) have isolated pure cannabinal having the formula $C_{21}H_{26}O_2$ from the crude cannabinal of previous workers. The pure cannabinal is a colourless oily liquid and forms with *p*-nitrobenzoyl chloride a crystalline compound, cannabinal-*p*-nitrobenzoate. It is a highly toxic substance and produces convulsions and death in a rabbit if injected as a 0.5 per cent acetone solution in a dose higher than 2 mgm. per kilo of body weight. From the same crude cannabinal they have further isolated a non-crystalline *p*-nitrobenzoate of cannabinal which on hydrolysis gives also an oily liquid (almost colourless) but this oil when injected in the same way in a rabbit in doses up to 5 mgm. per kilo of body weight produced profound sleep followed by death but no convulsions. In doses of 5 mgm. or more, the animal first got sleep and then died after convulsions. The fraction of pure cannabinal which gives the crystalline derivative does not produce the characteristic anæsthesia of the cornea (*Gayer effect*) which was first demonstrated by Gayer by injecting subcutaneously in rabbits, cats and mice an extract of the cannabis resin. On the other hand the fraction of cannabinal which neither gives the crystalline derivative nor produces convulsions but produces sleep, gives the Gayer

test in a dose of 0.25 mgm. per kilo of body weight. It is, therefore, evident that the resin of *C. sativa* contains at least two different toxic principles.

The fraction producing the crystalline derivative loses its toxicity in about 3 days when exposed to the air while the other fraction retains at least 25 per cent of its toxicity even after exposure for 6 months under the same conditions.

Extraction of Cannabis Resin from the Urine, Stomach Contents, etc.—The acid ether extract from the Stas-Otto process contains the resin which can be identified by some of the well known chemical tests described below. As cases of fatal poisoning by Cannabis have so far not been recorded, no investigation appears to have been made by any toxicologist to find out if the toxic principles are retained in the different organs of the human body. There is, however, no doubt that some active constituents of the Cannabis resin which give the characteristic chemical tests, are eliminated in the urine. By actual experiments on horses carried out with the help of the Royal Calcutta Turf Club by feeding the animal with *bhang*, we have found that the acid ether extract from the urine gives some of the specific tests described below.

Tests for Cannabis

(i) *Beam's Alkali Test*.—Purify the extract, if necessary, with animal charcoal for removing the colouring matter, particularly the chlorophyll of the crude drug. Evaporate to dryness in a porcelain basin and add a few drops of alcoholic potash (5—10 per cent)—when a violet colour gradually develops which may be hastened by warming.

This test may also be carried out in the following way: Dissolve the residue in a few drops of petroleum ether, soak a piece of filter paper in this solution and allow it to evaporate. Add a drop of alcoholic potash to the dry paper—the violet colour appears more rapidly.

(ii) *Beam's Acid Test*.—Dissolve the residue in a few drops of petroleum ether and add a few drops of absolute alcohol saturated with dry HCl gas—a red colour is produced.

N.B. Galenical preparations such as tincture of *Cannabis indica* often fail to give this test.

(iii) *Bouquat's Test*.—Dissolve the purified (with animal charcoal) residue in a few drops of acetone, add a few drops of freshly prepared mixture of conc. H_2SO_4 (2 vols.) and absolute alcohol (3 vols.)—the mixture darkens and a cherry red colour develops in about an hour. On adding a few drops of water, the colour disappears and the mixture becomes opalescent.

(iv) *Aldehyde Test*.—To the residue in a porcelain basin add a few drops of the reagent (prepared by dissolving 1 g. of para-dimethylamino-benzaldehyde in 100 c.c. of alcohol and then adding 20 drops of conc. H_2SO_4) and evaporate the mixture to dryness—a bright violet colour is produced.

N.B. It may be noted that all the colour tests described above give satisfactory results if applied to the resin extracted directly from *ganja* and *charas* or from their preparations containing the resin in appreciable amounts. The first three tests produce only faint colour reactions with *bhanga* which, however, is best detected by microscopic examination.

(v) *Microscopic Examination*.—This is the most reliable test for *bhanga*, *ganja*, *charas*, *modak*, etc., if the drug is present in its crude state, i.e., if the botanical structures were left undigested and intact in stomach contents, vomit, etc. The most characteristic structure is the retort-shaped, short, unicellular hair containing a cystolith at the root (see Fig. 55). It is found in fair number along with the long ordinary hair which possesses no cystolith. In some cases the hairs may be found in broken condition, the cystolith being detached from the tapering end of the hair. The examination is carried out in the following way: Pick up the suspicious particles and place on a slide, add a drop of dilute caustic soda solution, cover with a cover-slip and examine first under the $1/3''$ and then under the $1/6''$ objective when the morphological features of the hair will be seen distinctly.

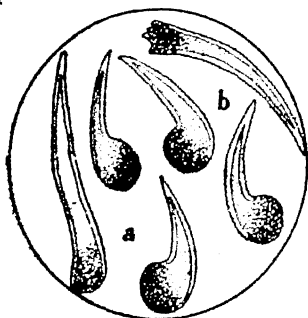


Fig. 55

The ganja hair appears smooth (a) while the hair found in charas shows warty surface (b).

In the case of *modak*, remove ghee, sugar and other substances by washing successively with petroleum ether, alcohol and hot water. Treat the residue with dilute alkali and then examine under the microscope.

Particles of *ganja* or *charas* mixed with tobacco or any other dry substance are readily identified by the following method: Remove the foreign matters as far as possible, rub the suspected material in a mortar with little water, add some chloral hydrate solution (50 g. of chloral hydrate and 20 c.c. of water), mix and transfer the mixture into a test tube. Boil for a few seconds when the finer particles will float on the surface. Remove a drop of the liquid containing the floating material with a lifter and examine under the microscope—the characteristic hair will be seen in fair number.

Effect of Oxidation on the Potency of the Active Principles of *Cannabis sativa*.—It is observed that *ganja* loses considerably its physiological potency on keeping and old samples may be absolutely free from its intoxicating properties for which it is smoked but the resin obtained from such samples gives the specific chemical tests all the same. For assaying the potency of *ganja* or *charas* feeding experiments on dogs hitherto considered most reliable are now found open to serious objections, the most important of which is the want of uniformity of signs and symptoms in all cases of *ganja* or *charas* intoxication. A veteran *ganja* smoker is on the other hand found to be a competent judge of the potency of these drugs. He would express his opinion on the potency of a sample of *ganja* or *charas* in terms of 'annas', sixteen annas being the 100 per cent potency, and detect a difference of two annas, i.e., about 12 per cent, without much difficulty. This fact is taken advantage of in provincial excise laboratories for bio-assay of *ganja* and *charas*.

It is claimed by certain workers that the specific rotation of the petroleum ether or carbon tetrachloride extract

of these drugs gives a definite indication of its potency but we found it useless in our experience.

4. Jequirity or Indian Liquorice. *Abrus precatorius* Linn.

This climbing plant belongs to the N.O. Leguminosæ and grows wild all over India. Its seeds are egg-shaped with a beautiful scarlet colour having a deep black spot at its upper end which is attached to the fruit. It is known as *kunch* in Bengal and *rati* or *gumchi* in Northern India and *gundamani* in Madras. The seeds are used by Indian goldsmiths for weighing silver and gold, each seed weighing on an average about $1\frac{1}{4}$ grains. White and brown seeds are also found.

The kernel of the seed is usually employed for killing cattle by professional cattle poisoners. It is made into a paste and then rolled between the fingers to form small needles or *sui* which become quite hard on drying in the sun. These needles are inserted in the soft tissues of the cattles, e.g., in the perineum, udder, etc. It is also used for homicidal purposes by holding the *sui* between two fingers and then slapping on the cheek or any other part of the body in such a way that the needle is inserted in the subcutaneous tissue. After a few hours it produces inflammation, pain and possibly some necrosis on the site, and subsequently general toxæmia and death.

A small particle of the kernel if put in the lower conjunctival sac of the eye for a few seconds, it produces conjunctivitis. This property is taken advantage of by those who want to shirk military or other duties. A few cases of malingering of this type had lately been detected in the Army.

Active Principles of *Abrus precatorius*

The toxic principle of *Abrus* is a toxalbumin or phyto-toxin called *abrin*. *Abrine*, another substance originally believed to be an alkaloid, has now been proved to be α -methylamino- β -3-indolyl propionic acid. Certain other principles having agglutinating action on red blood cells, and some enzymes and organic acids are also found in *Abrus*.

Abrin resembles ricin (present in castor oil seeds), croton (present in croton seeds) and snake venom in their action on the human system. When taken by the mouth, abrin undergoes digestion and produces no toxic effect if the mucous membranes of the alimentary system are uninjured. If it is injected subcutaneously or applied to a wound, it is rapidly absorbed and produces acute symptoms of poisoning and death.

Extraction of the Poison.—As abrus is not given with food or drink, its detection in stomach contents, vomit, urine, etc., is not called for. The particles of *sui* may be found embedded in the tissue where it is inserted and the examination of the tissue suspected to contain the particles is required in such cases. As abrin is an albuminous substance, the usual process of extraction by alcohol and other organic solvents is not applicable. In a case of abrus poisoning, the suspected particles are to be picked up carefully and an aqueous solution of the same is to be tested physiologically. No chemical test has yet been worked out.

Tests for Abrus

(i) If the original *sui* or a fragment is available, insert it under the skin of the inner side of the thigh of a fowl— inflammation sets in a few hours followed by necrosis at the site of insertion and the fowl dies in about 24 to 36 hours.

(ii) If *sui* is not detected, take a small portion of the tissue suspected to contain the particles of abrus, make into a paste with a few drops of water, dilute it to one c.c. with water and inject a portion of the solution into a healthy fowl as described above. The bird will develop the characteristic signs and symptoms if abrus is present. Dissect carefully the site of injection and look for necrosis of the tissue.

(iii) If small non-toxic doses of abrin are injected at regular intervals into a susceptible animal, an antibody is formed in its blood and the animal becomes immune against a toxic dose of the poison. The blood serum obtained from this immunized animal acquires the property of giving a

precipitate if added in suitable dilution to a diluted solution of abrus. This *precipitin* test is carried out with the anti-abrin serum^{*} available in the market. This serum is also used in veterinary practice for the treatment of abrus poisoning if detected early.

N.B. As the *precipitin* test is a very delicate one requiring much skill and experience, it is best done by specialists in this line.

Extraction of Abrin from Abrus Seeds—Remove the seed envelope, rub the kernel in a mortar with 4 per cent NaCl solution in which abrin is soluble. Allow to settle and separate the NaCl solution of abrin. Repeat the process 2 or 3 times more. Filter the combined extracts and concentrate in vacuo. Acidify with acetic acid and saturate with NaCl to precipitate the abrin. Separate the precipitate and purify it by dialysis in a parchment dialyzer for several days. Finally dry the residual abrin in vacuo over sulphuric acid when an amorphous powder is obtained.

Agglutination Test for Abrin—Take 2 c.c. of defibrinated blood (not diluted) in a small test tube and add to it 1 or 2 drops of abrin solution (0.1 g. dissolved in 10 c.c. of 4 per cent NaCl solution), the red blood corpuscles agglutinate into a mass resembling sealing wax.

The agglutination is also observed under the microscope by mixing a trace of the abrin solution with a drop of defibrinated blood, the clumping of red blood cells takes place in a few minutes.

Abrin, in common with other toxalbumins such as ricin and ~~cap~~otin, coagulates milk.

5. Croton. *Croton tiglium* Linn

Croton belongs to the N.O. Euphorbiaceæ and grows all over India. The seeds as well as the oil extracted from them are highly poisonous and are used in indigenous systems of medicine. Cases of poisoning by croton are mostly homicidal and occasionally accidental. It is sometimes used as an abortifacient. The poisonous action of the seed

is due to its oil as well as the toxalbumin *croton* present in it. The seed is known as *joypal* in Bengal, *jamalgota* in Northern India and *nervalam* in the Madras Presidency.

Active Principles of Croton

Croton, a toxalbumin or phytotoxin, resembles abrin in its agglutinating action on red blood corpuscles (see p. 534). A glycoside, *crotonoside* (6-amino-2-hydroxy purine-*d*-riboside), has lately been isolated from the seed. *Croton* oil, extracted from the seed, is a powerful drastic purgative. It contains a *vesicating resinous substance*, crotonoleic acid, palmitic, tiglic (methyl crotonic acid), etc. Croton oil dissolves readily in absolute alcohol, ether, chloroform, benzene, etc. The solubility of this oil in alcohol renders it suitable for extraction from viscera and other materials by the Stas-Otto process.

Extraction of Croton—In poisoning by croton seeds, it is not necessary to make any attempt for the isolation and detection of croton. The detection of the oil which is easily extractable serves the purpose quite well. The extraction of croton oil is carried out in the following way: Proceed with the Stas-Otto method of extraction. The acid ether extract (stage c. p. 428) which contains the oil may be submitted to the tests described below.

If there is any definite history of croton poisoning, the method of direct extraction of the oil with ether may be employed instead of going through the time-consuming Stas-Otto process. For direct extraction with ether, acidulate the material (stomach contents, vomit or viscera) with tartaric acid and add slowly with stirring a sufficient quantity of ether to cover the material. Allow the ether layer to separate and decant. Repeat the process several times when the oil will be completely extracted. Evaporate the ether extracts and apply the specific tests to the residue.

Tests for Croton Oil

(i) Dissolve the oil by vigorous shaking in twice the volume of absolute alcohol. Layer the alcoholic solution

over an equal quantity of caustic soda solution (about 40 per cent) or saturated alcoholic potash in a test tube, a brownish red or reddish violet ring develops at the junction of the two liquids if the oil extracted is pure and present in sufficient quantity. The reaction is hastened by warming.

(ii) To the residue (ether extract) add drop by drop an alcoholic solution of *p*-dimethylaminobenzaldehyde (1 per cent solution in 95 per cent alcohol containing one per cent conc. H_2SO_4), a transient red colour may be noticed. On adding 1 or more drops of the reagent, a transient pale blue colour is seen. Evaporate to dryness, the residue becomes brownish red or purple which changes to pale blue colour on adding a little more of the reagent (Bamford's Poisons, 1940). This test also depends on the amount of the oil extracted.

(iii) *Physiological Tests*.—(a) Apply a small drop of the oil to the skin of the inside of the forearm or gently rub it, signs of irritation will be noticed after a few hours and vesication follows next morning.

(b) If the amount of the oil is very small, no irritation or vesication is likely to be produced. In such cases, apply a trace of the residue to the tip of the tongue and gently rub it with the tip of the finger, in a few minutes a burning sensation of the tip of the tongue with a peculiar sense of dryness of the throat will develop.

(c) *Agglutination Test for Croton*.—For extraction of crotin and performance of the agglutination test with crotin, proceed as in abrin (p. 533). Crotin also coagulates milk.

6. **Madar.** *Calotropis gigantea* R. Br. and *C. procera* R.Br.

Calotropis is known in this country from ancient times for its medicinal and other properties. It is traced to the Vedic literature; the sacred *soma* juice is believed to be the fermented sap of a species of *calotropis*. The vernacular name *madar* comes originally from its Sanskrit name *mandara* or *arha*. It is known as *akanda* in Bengal and *ak*, *akwan* or *madar* in Northern and Western India and *cruhku* in Tamil and *mandaramu* in Telegu.

The plant belongs to the N.O. Asclepiadaceæ and grows wild all over India. The common purple flowered variety is *C. gigantea* and the white flowered one is *C. procera*, both of them having the same chemical and physiological properties. The milky juice obtained from the leaves, stems, barks, etc., gives a kind of gutta-percha which is not of any commercial importance. The powdered bark of the root and particularly the milky juice from the leaves and stalks are used for various criminal purposes, viz., for homicide, suicide and infanticide and also as a cattle poison but most commonly for procuring abortion. The milky juice is a powerful irritant both internally and externally; when applied to the skin, it produces redness and even vesication. The small stems are often used as *abortion sticks* which are inserted into the os uteri. The root, particularly of the white flowered *calotropis*, is a powerful poison to cobras and other poisonous snakes which cannot stand even the smell of it. It is always carried by the snake charmers of Bengal to control the newly caught and unruly cobras. We demonstrated the action of the root on an enraged cobra which when going to strike with its expanded hood, shrank its hood at once and retreated to its shelter on smelling a small piece of the root held in front of its head. Both *procera* and *gigantea* are used in Africa as arrow poison and hence also known as "African arrow poison."



Fig. 56

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Active Principles of Madar

Hesse, Reicheneder and Eysenbach (Annalen, 1938) isolated from *C. procera* and *gigantea* *Uscharin* (0.45% of the latex), *Calotoxin* (0.15%) and *Calactin* (0.15%). The formula of *Uscharin* is stated to be $C_{31}H_{41}O_8NS$. m.p $265^{\circ}C$ (with decomposition), fatal dose 0.5 g/g. in

frogs. *Calotoxin* $C_{29}H_{40}O_{10}$, m.p. 244°C (decomposition), fatal dose 0.7 g. in frogs.

Chen, Bliss and Robbins (*J. Pharmacol.*, 1942) isolated from *C. procera* and *gigantea* the principles *Calotropin*, *Calotoxin* and *Uscharin*, the toxicities of which compared to *Ouabain* as 100 are: *Calotropin* 83, *Calotoxin* 76 and *Uscharin* 58.

S. Rajagopal Naidu states in his annual report (1936) that the milky juice of *madar* is of acid reaction and clots on standing for sometime and gives a clear straw coloured serum. The clot gives a yellowish brown *resin* and a white crystalline substance $C_{27}H_{46}O_2$. The resin is highly poisonous but the crystalline substance is non-poisonous. The serum is also highly toxic.

N. Pitchandi (working with S. R. Naidu, 1946) has isolated from the serum of *C. gigantea* a white crystalline substance, free from sulphur and nitrogen and having the formula $C_{44}H_{36}O_8$ and m.p. 232°C (with decomposition) which he has named *gigantin*. It is soluble in alcohol but insoluble in water and is highly toxic—15 to 20 times as toxic as strychnine. The minimum fatal dose (intravenous) is 0.2 mg. per kilo in dogs, death taking place in 20-60 minutes, no convulsions. (Private communication).

Extraction of the Poisonous Principles of Madar—

(a) The extraction of the poisonous principles of *madar* from the powdered root-bark or the milky juice present in the quack remedies or from the dried juice present in the *abortion stick* is readily carried out by the following method; Reflux the material with absolute alcohol for about 2 hours. Filter and evaporate the filtrate to dryness and then apply the specific tests to the residue.

(b) In the case of viscera, stomach contents and vomit it is necessary to follow the Stas-Otto process of extraction, using absolute alcohol instead of rectified spirit in all its stages: Take the finely minced material and proceed up to the end of the stage (b) (see p. 427). Divide the combined alcoholic extracts into two parts A and B. Evaporate the part A almost to dryness and saponify the residue with

alcoholic potash and then extract with petroleum ether. Keep the alkaline alcoholic solution for ester test. Evaporate the ether extract to dryness. Take up the residue in chloroform and treat with a slight excess of digitonin dissolved in rectified spirit or ether. Evaporate the mixture to dryness and extract with ether. The ether extract on evaporation may give a crystalline residue if madar is present, but the usual impurities coming from tissues almost always interfere with the production of a crystalline precipitate.

Tests for Madar

For the residue from (a).—(i) *Ester Test*.—To a portion of the residue add 1 or 2 c.c. of absolute alcohol and a little sulphuric acid (1:1) and gently heat to boiling, the fruity odour of an ester (like that of a ripe jack fruit) is recognized if madar is present.

(ii) To another portion add a few drops of conc. HCl, a bluish or greenish blue colour develops which is discharged on keeping or heating.

(iii) To another portion add a drop or two of conc. H_2SO_4 , a green colour appears which changes to brown or purple. With dilute acid, the original colour may be pink.

N.B. The ester test is more dependable than the colour reactions which may be greatly masked by the presence of impurities.

(iv) If the original alcoholic extract is purified and allowed to evaporate spontaneously, the residue may give some cauliflower like masses or nodules which are believed to be characteristic of madar juice (Black's Test).

For the residue from (b).—(i) Take a small amount of the residue on a porcelain plate, add a drop or two of conc. H_2SO_4 , a red colour develops which changes to purple on adding chloroform and acetic anhydride (Naidu's Test).

(ii) Evaporate the alkaline alcoholic solution nearly to dryness, take up in absolute alcohol and filter. To the filtrate add some conc. HCl, warm and allow to stand, the characteristic odour of the ester is recognized if madar juice is present. Slight dilution with warm water makes the odour more readily perceptible.

(iii) *Physiological Test*.—Take the second part (B) of the alcoholic extract and evaporate to dryness. Dissolve the residue in water acidulated with acetic acid and filter. Treat the filtrate with lead acetate and filter off the precipitate. Pass H_2S through the filtrate to remove the excess of lead and filter off the lead sulphide. Evaporate the filtrate to dryness on the water bath and extract the residue with absolute alcohol. Filter again and evaporate to dryness. Dissolve a portion of the residue in a few drops of water and inject into the dorsal lymph sac of a frog—in a few minutes convulsions set in which are followed by paralysis and death. The frog usually shows a bloated appearance.

7. *Cleistanthus collinus* Benth.

The plant *C. collinus* belongs to the N.O. Euphorbiaceæ and grows in the hilly tracts of Santal Perganas and Singbhum in Bihar and in Orissa, Madras and possibly in other provinces of India. It is known as *oduvan* in Madras and *karajuri* or *pasu* in Bihar. The plant has been grown in the Botanical Gardens of Sibpur, Calcutta. The leaves and barks are commonly used as a paste or decoction for homicidal and suicidal purposes and also as a fish poison. In autumn, the leaves falling into tanks have been known to cause death of a large number of fishes in tanks situated in jungles where *oduvan* grows.

Active Principles of *Cleistanthus Collinus*

Hitherto no serious attempts had been made to isolate the active principle of this plant and to study its chemistry and toxicology. Naidu and his colleagues have isolated a glucoside having the formula $C_{22}H_{34}O_{12}$ and termed it *oduvin*. It is a yellowish white crystalline substance (m.p. $192-194^{\circ}C$), readily soluble in alcohol and chloroform but sparingly soluble in ether and water (Jour. & Proc., Inst. Chem., 1944). About 0.75 mg. of *oduvin* kills a frog weighing about 10 g. in a few minutes with paralysis.

Extraction of the Poisonous Principle.—Evaporate the acid alcoholic extract (stage 'a' of the Stas-Otto process) to a pasty consistency, dissolve it in warm water and filter. Alkalinize the filtrate with sodium carbonate and extract

with ether-chloroform mixture (1:3). Evaporate the ether-chloroform extracts to dryness. The residue contains the active principle *oduvin*.

The acid ether extract of the Stas-Otto process also yields this poisonous principle but it undergoes some change during extraction although it retains its toxic action to frogs.

Tests for Oduvin

(i) Add a drop of conc. H_2SO_4 to *oduvin*—a blue colour changing to mauve or permanganate is produced.

(ii) Add a drop of conc. HNO_3 —an evanescent a green colour develops which changes to brick red. Fuming HNO_3 gives an immediate vermilion colour resembling that of brucine.

(iii) The acid ether extract of the leaves gives a green colour with conc. HCl and a purple with conc. H_2SO_4 while alkaline ether extract does not give the green colour.

In some cases the poisonous principle is decomposed and cannot be identified by the colour tests described above. In this condition, the microscopic examination of the particles of leaf usually found in the stomach contents, is of great value in identifying the poison. The diagnostic features of the leaf have been described in the original paper referred to above.

8. Marking Nut. *Semecarpus anacardium* Linn.

The marking nut tree belongs to the N.O. Anacardiaceæ and grows all over India. The nut contains a fleshy pulp from which is expressed a dark brown, oily, acrid juice which turns black when mixed with lime and exposed to air. It is used by washermen for marking linen and is known as *bhela* in Bengal, *bhilawan* or *bhela* in Northern India, and *bhiba* or *biba* in the Bombay Presidency. It is used in the indigenous systems of medicine both internally and externally. The juice is irritant and possesses vesicating action. Poisoning by the marking nut juice is usually homicidal and occasionally accidental. It is also used for procuring criminal abortion by applying the bruised nut to the os uteri. Cases have been reported here in which the pure juice or the juice mixed with oil was introduced into the vagina as a

punishment for infidelity or for taking vengeance by a woman on another woman for alienating the love of her paramour. In all such cases severe vesication and ulceration followed causing grievous hurt.

Active Principles of Marking Nut. —No systematic investigation has been carried out on this interesting poison.

The following constituents have been isolated by Pillay and Siddiqui (*Jour. Ind. Chem. Soc.*, 1931) from the juice of the pericarp of the nut:

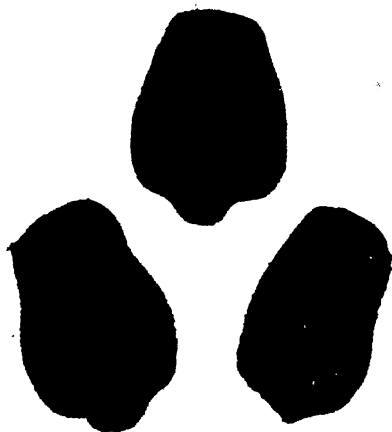


Fig. 57.

(i) *Semecarpol*, a monohydroxy phenol, which boils at 185° — 100°C (at 2.5 mm. pressure) and forms only 0.1 per cent of the extract.

(ii) *Bhilawanol*, an o-dihydroxy compound which boils at 225° — 226°C (at 3 mm. pres

sure) and forms about 46 per cent of the juice.

(iii) A tarry non-volatile corrosive residue forming about 18 per cent of the nut.

(iv) Fatty oils, tannic acid and other acids.

Extraction of the Poisonous Principle.—The extraction is carried out by the Stas-Otto method in the following manner: Take the material (finely minced viscera, stomach contents, vomit, stained cloth, etc.) and proceed with the Stas-Otto process up to the stage of the evaporation of the alcoholic extract. Take up the alcoholic residue in hot water, faintly acidify with dil. H_2SO_4 and extract with petroleum ether. Evaporate the ether extract to dryness. The residue contains the vesicating principles of the marking nut juice.

Tests for Marking Nut

(i) Dissolve a portion of the residue in a little alcohol

and add a few drops of alcoholic potash—a bluish green or green colour develops.

(ii) Dissolve another portion in a little alcohol and add a few drops of basic lead acetate solution—a greenish black precipitate is formed.

(iii) *Physiological Test*.—To the residue add a drop or two of olive oil, mix them well and rub a drop of the mixture into the skin (about $\frac{1}{4}$ th of an inch in diameter) of the inner side of the forearm and keep it covered with a bandage for about 4 hours—a blister will form next day or after 48 hours. The test may be performed on the abdominal skin of a cat or a rabbit after depilation.

As these blisters are very painful and tend to spread over the surrounding area, great care should be taken to perform this test. If it is suspected that the reaction will be strongly positive, the residue should be diluted with 2, 3, or more parts of olive oil before it is applied to the skin, when only an erythema or redness of the skin will be noticed.

N.B. The vesicating action of the active principle of the marking-nut juice is destroyed by caustic potash. If the petroleum ether residue is mixed with cold caustic potash solution, allowed to stand overnight, reacidified with dil. HCl and then re-extracted with petroleum ether, this residue if applied to the skin as before will produce no blister. This property distinguishes it from other vesicating principles which are not affected by caustic potash.

(iv) For identification of the stain on blisters or to determine the cause of vesication, remove the epidermis of the blister and extract it with absolute alcohol. To the alcoholic extract add a few drops of alcoholic potash—a bluish green colour will develop.

CHAPTER XXXVI

Inorganic Poisons; Nitrites. Iodine. Phosphorus. Arsenic and its Organic Derivatives. Mercury. Antimony and its Organic Derivatives. Barium. Lead. Copper. Chromium. Manganese.

1. Nitrites

Poisoning from nitrites particularly from sodium nitrite, which is used in dyeing and textile industry, is fairly common, and cases of accidental and suicidal poisoning have been reported from Bengal, Madras and other provinces. The victims are usually the weavers in the countryside where they dye their own yarns. The first authentic case of suicidal poisoning by sodium nitrite was reported by the Chemical Examiner, Bengal, in 1930.

The nitrites act on the blood and convert oxyhæmoglobin into methæmoglobin and also act as irritant poisons on the mucous membrane of the stomach.

Extraction of Nitrites.—Small traces of nitrites may occur normally in stomach and intestinal contents owing to reduction of the nitrates present in food and water by bacterial action. Hence detection of a mere trace in the tissues or stomach contents should not be regarded as an indication of nitrite poisoning. In such cases a quantitative determination of the nitrite is called for.

The nitrites may be extracted from tissues and other materials by the following method: Digest 2 or 3 times with warm water, allow to settle and filter. Repeat the filtration to obtain a clear and colourless filtrate. Decolourize by animal charcoal if necessary. The filtrate containing nitrites and possibly some nitrates is submitted to the usual tests for nitrites.

Tests for Nitrites

(i) **Starch-Iodide Test.**—To a portion of the extract, slightly acidulated with dil. H_2SO_4 (1:2), add a few drops

of KI solution and a drop or two of starch solution—a blue colour appears at once. Instead of using solutions of KI and starch, a starch-iodide paper may be used.

(ii) *Bismarck-Brown Test*.—To a portion, acidulated with dil H_2SO_4 , add a few drops of 0.5 per cent solution of metaphenylenediamine hydrochloride (decolourized before use by animal charcoal)—a brown colour (Bismarck Brown) develops.

(iii) *Griess-Ilosvay Test*.—Two reagents are required for this test: (a) 0.3 per cent sulphanilic acid solution in 20 per cent acetic acid, and (b) 0.07 per cent solution of α -naphthylamine in 20 per cent acetic acid.

To a portion of the extract add 5-10 drops of sulphanilic acid solution and mix and then add 5-10 drops of α -naphthylamine solution and mix—a red colouration is produced. This is a form of diazo reaction and is a very delicate test for nitrites. This reaction is used for quantitative determination.

(iv) *Moir's Test*.—To a portion of the extract add 2 c.c. of *p*-nitraniline solution (prepared by dissolving 0.3 g. of *p*-nitraniline in 8 c.c. of conc. HCl and diluting to 100 c.c.) and heat to about 50°C for a few minutes, then add 2 c.c. of α -naphthol solution (prepared by dissolving 0.4 g. of α -naphthol, 0.22 g. of NaOH and 2 g. of sodium acetate in 100 c.c. of water)—an orange red precipitate of *p*-nitrobenzene-azo- α -naphthol is formed which dissolves in little excess of NaOH solution giving a beautiful violet colouration.

This test may also be used colorimetrically for the quantitative determination of nitrites.

Quantitative Determination

(1) *For Materials other than Viscera*.—Weigh out the material (stomach contents, vomit or other substances), mix with enough nitrite-free water to make it a thin gruel, acidulate with little excess of acetic acid and distil in a current of CO_2 (to prevent oxidation of HNO_2 to HNO_3). Collect the nitrous fumes in a receiver containing some water in which the end of the condenser is dipped. Measure the distillate and take an aliquot portion in 50 c.c. Nessler cylinder. To this add 1 c.c. each of the Griess-Ilosvay reagents described above, mix well and make up to 50 c.c. and wait for 30 minutes by which

time the maximum intensity of the colouration is obtained. Match the depth of this colour against a series of Nessler tubes containing known quantities of standard sodium nitrite solution treated exactly in the same way as the unknown was treated.

(2) *For Viscera*.—Weigh out the finely minced tissue in a beaker, mix with some nitrite-free water, neutralize with dil. NaOH solution if the reaction is acid and transfer to a 500 c.c. volumetric flask. Add sufficient quantity of nitrite-free hot water (heated to about 80°C) to give it a consistency of a thin gruel, place the flask on a steam bath for about 2 hours with frequent shaking. Add sufficient saturated solution of mercuric chloride to precipitate the proteins, mix, cool to room temperature, make up to 500 c.c. mark with nitrite-free water, and mix again. Filter and take an aliquot part of the filtrate (2 to 5 c.c. or more according to the preliminary test carried out to obtain an idea of the depth of colour produced) in a 50 c.c. Nessler tube, make up to 50 c.c. mark with nitrite-free water, add 1–2 drops of conc. HCl, 1 c.c. each of the Griess-Ilosvay reagents, thoroughly mix and allow the colour to develop for 30 minutes. In the meantime, measure out different quantities of the standard solution (0.2, 0.5, 1.0, 2.0 c.c., etc.) in a series of Nessler tubes and treat them in the same way as the unknown sample was treated. Set aside all the tubes for 30 minutes and match the depth of the pink colour of the unknown solution against the closest of the standard solutions. Calculate the result on the strength of the standard solution matched.

Preparation of the Standard Solution.—Dissolve 4.46 g. of chemically pure AgNO_3 in about 200 c.c. of nitrite-free water, add sufficient NaCl solution to precipitate AgCl , dilute to one litre, mix and allow to settle. Take 100 c.c. of the clear supernatant liquid and make up to one litre, using in each case nitrite-free water. Each c.c. of the last solution is equivalent to 0.002 mgm. of sodium nitrite.

2. Iodine

Poisoning from iodine is mostly accidental but cases of attempted suicide by swallowing tincture of iodine are not uncommon and some of them end fatally.

Extraction of Iodine and Iodides.—Iodine is eliminated from the system as iodides and rarely as free iodine in the urine. When it comes in contact with tissues or albuminous fluids it is fixed up partly by the alkali and partly by the proteins, and as such free iodine is rarely available for extraction. In the blood it is converted mostly into sodium iodide and is excreted in this form in the urine and faeces.

As the tincture of iodine contains free iodine, KI and alcohol, detection of appreciable amounts of KI along with

alcohol in the material under examination indicates poisoning by the tincture.

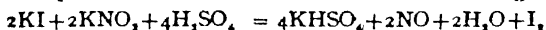
For extraction of free iodine from tissues, stomach contents, etc., mix the material with water (not necessary for the urine) and distil, the vapour being condensed in an ice-cooled condenser the end of which is dipped in a few c.c. of chloroform contained in the receiver. If free iodine is present in the distillate, chloroform takes it up and becomes pink coloured.

If no free iodine is detected, render the original material strongly alkaline with NaOH, mix with water and filter. Add some solid NaNO_3 to the filtrate, mix thoroughly and evaporate the mixture to dryness in a platinum crucible. Carefully incinerate the residue at a low temperature. Dissolve the ash in water, acidify with dil. H_2SO_4 , add some solid NaNO_2 and chloroform and shake vigorously for a few seconds, chloroform takes up the free iodine liberated from the iodides and is coloured pink.

Tests for Iodine

(i) The purple colouration of chloroform is itself a test for iodine. To 1 or 2 drops of this solution add 5 c.c. of water, 2 drops of starch solution and shake, a blue colour appears.

(ii) In the case of *urine and stomach washings*, the following procedure may be followed: Acidify with H_2SO_4 , add a few c.c. of freshly prepared solution of sodium or potassium nitrite, some chloroform or carbon disulphide and shake vigorously when the free iodine liberated from the iodides will pass into the immiscible solvent and tinge it pink.



Quantitative Determination of Iodides

As chlorides are normally present in the urine and tissues, the ash of the material weighed out for determination of iodides contains both iodides and chlorides. As such the total halogens (chlorides and iodides) should be determined first and then in another portion, the amount of chlorides has to be determined after removing the iodides by boiling with H_2SO_4 and NaNO_2 as described before. The difference between these two values gives the amount of the iodide. Proceed in the following manner:

Volhard's Method.—Dissolve the ash of a known quantity of the material in water, filter and make up to a known volume. Take an aliquot portion (say 20 c.c.) in a stoppered volumetric flask (500

c.c.), dilute to 200—300 c.c. and add drop by drop $N/10$ $AgNO_3$ solution with vigorous shaking until the precipitate collects together and the supernatant liquid appears colourless. As long as the solution appears milky, the precipitation is not complete. Finally, add a little more $N/10$ $AgNO_3$ and again shake. Render the solution strongly acid with about 10 c.c. of HNO_3 (free from lower oxides of nitrogen; it may be prepared by boiling 100 c.c. of HNO_3 with 25 c.c. of water till it is perfectly colourless) and mix. Make up to 500 c.c. mark and filter through a dry filter, rejecting the first 10 or 20 c.c. of the filtrate. Take 100 c.c. of the filtrate, add 5 c.c. of a clear saturated solution of iron alum (ferric ammonium sulphate) and titrate the excess of $N/10$ $AgNO_3$ with $N/10$ potassium thiocyanate solution till a permanent red colouration is observed. The total amount of $N/10$ $AgNO_3$ added minus the amount of $N/10$ KCNS required for titration gives the amount of $N/10$ $AgNO_3$ taken up by the chlorides and iodides in 100 c.c. Calculate for 500 c.c. which is equivalent to 20 c.c. of the original solution.

Take another portion (20 c.c.) of the original solution (i.e., of the ash dissolved in water and filtered), dilute with water to 200-300 c.c., acidify with dil. H_2SO_4 (2-3 c.c.), add 0.5 g. to 1.0 g. of solid KNO_3 and boil the solution until entirely colourless which takes place in about 40 minutes. Then add, drop by drop, $N/10$ $AgNO_3$ and proceed in the same way as described above. The amount of $N/10$ $AgNO_3$ minus the amount of $N/10$ KCNS required for titration gives the amount of $N/10$ $AgNO_3$ taken up by the chlorides. Calculate for 500 c.c. which is equivalent to 20 c.c. of the original solution. The difference between the first result (for chlorides and iodides) and the second result (for chlorides) gives the amount of $AgNO_3$ combined with the iodides.

Since 1 c.c. of $N/10$ $AgNO_3$ = 0.0127 g. of iodine, and 1 g. of iodine = 1.307 g. of KI, the factor 0.0166 (i.e., 0.0127×1.307) multiplied by the amount of $N/10$ $AgNO_3$ would give the amount of iodide (as KI) present in the aliquot portion (20 c.c.) of the solution taken for determination. Calculate the total amount of iodides present in the known quantity of the material.

3. Phosphorus

Poisoning by phosphorus may be acute or chronic; the former is of toxicological importance while the latter, which is of industrial or occupational origin, is still unknown in this country.

This ordinary phosphorus or yellow phosphorus, as it is called, is poisonous but its allotropic form, red phosphorus, is non-poisonous and does not oxidize in the air. Poisoning from yellow phosphorus is rare in this country but cases of poisoning in children by eating certain 'fire-works' are frequently met with although they are manufactured with non-toxic red phosphorus. The cause of such unforeseen

accidents is that certain types of 'fire-works' are manufactured with red phosphorus containing a small proportion of yellow phosphorus added to it. Phosphorus is absorbed in sufficient quantities even from aqueous suspensions and produces fatal results, the absorption being facilitated by the presence of fatty materials and also of bile.

Yellow phosphorus is a translucent, nearly colourless solid, having a consistency of that of bees wax but on keeping under water it acquires a yellow colour. It is insoluble in water but soluble in 400 parts of alcohol, 102 parts of ether, 40 parts of chloroform, 32 parts of benzene and 0.9 part of carbon disulphide at 25°C . It is sparingly soluble in fixed oils but readily in essential oils. Its garlic-like odour is very characteristic.

Tests for Phosphorus

(i) *Scherer's Test*.—Take some of the finely minced tissues, particularly the stomach with its contents, or some vomit or stomach washings in a conical flask (about 500 c.c. capacity) and cover with cold water if enough fluid is not present. Add a few c.c. of cadmium sulphate solution and acidify with dilute sulphuric acid. Close the flask with a cork in which two slits and a groove (to allow expansion of air) have been cut. Insert in the slits two pieces of filter paper, one soaked in 5 per cent AgNO_3 solution and the other in an alkaline solution of a lead salt (prepared by adding caustic soda to 5 per cent lead acetate solution until the precipitate first formed is redissolved) taking care that the papers touch neither each other nor the sides of the flask (see Fig. 58). Heat the flask in a water bath at a temperature of about $40^{\circ}\text{--}50^{\circ}\text{C}$ in a dark room to avoid the effect of light on the AgNO_3 paper. If the silver paper darkens while the lead paper remains unaffected, phosphorus may be present; if it does not darken, phosphorus is absent. If both papers darken, the test is inconclusive, since the change in

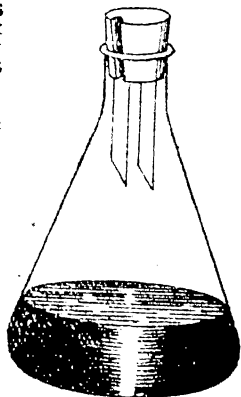


Fig. 58

colour may be due to H_2S or to both phosphorus and H_2S . Addition of cadmium sulphate solution fixes up the H_2S as cadmium sulphide and thus prevents it from interfering with the test. The negative Scherer test is of more value than the positive test, as formic acid, formadehyde, etc., produce blackening of the silver nitrate paper.

(ii) *Mitscherlich's Test*.—It is a confirmatory test and depends on the fact that yellow phosphorus volatilizes in steam and becomes luminous in contact with air.

Take sufficient material in a distilling flask and dilute with water to bring the consistency of the contents of the flask to that of a thin gruel and acidify with dil. H_2SO_4 .

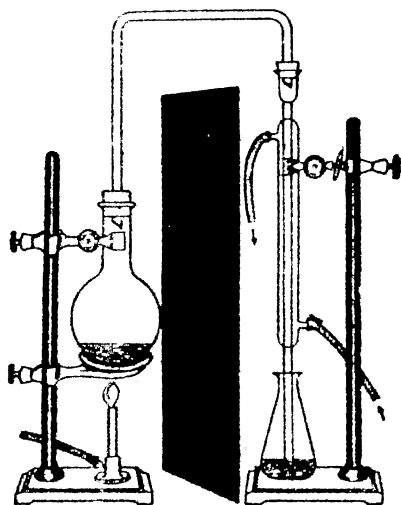


Fig. 59

Add some pure ferrous sulphate to eliminate to a certain extent the disturbing influence of oxidizing agents if any (for instance, the usual oxidizing agents present in match-heads) and some ferric chloride solution to fix up the H_2S if any (as indicated by blackening of the lead paper in Scherer's Test) and to prevent it from passing over to the distillate. Now heat the mixture over a wire gauze or on a sand bath and distil in a dark room, the distillate being received in a

little water in which the end of the condenser is dipped. It is desirable to place a black screen of card board or tin sheet between the condenser and the burner (see Fig. 59). As the distillation proceeds, a beautiful phosphorescence will be noticed in the condenser especially at its upper end and some times it appears in the form of a luminous ring.

N.B. The following substances have been known to interfere with this test by inhibiting the phosphorescent effect: Ethyl and methyl alcohols, ether, ethyl acetate, benzene, turpentine and other essential oils, carbolic acid and other volatile phenols, chloroform, chloral hydrate, sulphuretted hydrogen, ammonia, sulphur dioxide, iodine, bromine, chlorine, salts of Hg, Cu, and Ag and a few other compounds.

If no phosphorescence becomes visible, continue distillation until the greater part of the liquid has distilled over. Add a few drops of conc. HNO_3 to the distillate and gently boil under a reflux to oxidize any phosphorus which might have come but did not show any phosphorescence owing to the presence of an interfering substance. Concentrate it to only a few c.c. by heating on the steam bath and test for phosphoric acid.

Tests for Phosphoric Acid

(i) To a portion of the concentrated solution add a few drops of conc. HNO_3 and some ammonium molybdate solution and boil—a yellow crystalline precipitate of ammonium phosphomolybdate $12 \text{ MoO}_3(\text{NH}_4)_3\text{PO}_4$ is formed.

(ii) Magnesia mixture, containing equal volumes of MgCl_2 , NH_4Cl and NH_4OH solutions (about 10 per cent each), gives a white crystalline precipitate of $\text{Mg}(\text{NH}_4)\text{PO}_4$, which is insoluble in ammonia but soluble in acids. Instead of magnesia mixture, a solution of MgSO_4 and ammonia may also be used.

Quantitative Determination

The quantitative determination of phosphorus in tissues and other materials is a time consuming process and is not ordinarily necessary. Simple detection of phosphorus serves the purpose. For details of quantitative determination books on advanced toxicological chemistry may be referred to.

4. Arsenic

Poisoning from arsenic is very common in this country. In 1939, the percentage of fatal cases of human arsenic poisoning in different provinces was as follows: North Western Frontier Province 43, U.P. and C.P. 19, Punjab 17, Bengal 14, Madras 6, and Bombay 4. The fatal cases of arsenic poisoning are mostly homicidal and a few are suicidal and accidental. Most of the fatal cases of cattle poisoning (by professional cattle poisoners) are due to arsenic.

The following *arsenic compounds* have been known to cause acute poisoning:

- (i) White arsenic, Arsenious oxide, Arsenic trioxide, As_2O_3 .
- (ii) Yellow sulphide of arsenic, Orpiment, As_2S_3 .
- (iii) Red sulphide of arsenic, Realgar, As_2S_2 .
- (iv) Paris green, Schweinfurt green, Copper acetoarsenite $(\text{C}_2\text{H}_3\text{O}_2)_2\text{Cu}_3\text{Cu}(\text{AsO}_2)_2$ containing 58.5% of As_2O_3 , used in killing mosquito larvæ by spraying over cesspools, marshes, etc.
- (v) Arsine [actually diethylarsine $\text{AsH}(\text{C}_2\text{H}_5)_2$] produced by the action of common moulds such as *Penicillium brevicaulis* on arsenic compounds contained in pigments (wall-papers), etc.
- (vi) Insecticides and vermin killers, e.g., 'Sheep-dip', 'Rough on Rats', etc.—the latter containing 50–70 per cent of As_2O_3 .
- (vii) Medicinal preparations such as, Fowler's solution (which contains potassium arsenite), Salvarsan, Sulpharsenol, etc.

Of these, white arsenic accounts for 99 per cent of cases of arsenic poisoning in this country. Red and yellow sulphides of arsenic are insoluble and therefore harmless if pure, but commercial forms are always impure containing quite a large quantity of As_2O_3 . The sulphides are usually given for procuring criminal abortion but most cases end fatally. Poisoning by diethylarsine and arsenical insecticides or larvicides has so far not been reported in this country while cases of accidental poisoning by organic arsenicals such as salvarsan, are occasionally met with.

White arsenic is an ideal homicidal poison on account of the facts that being a colourless, tasteless and odourless compound it may be mixed with food or drink without rousing any suspicion, and that it produces signs and symptoms simulating those of cholera. It is slowly soluble in water and also slightly in alcohol and ether. It is soluble in dilute HCl and in solutions of alkali hydroxides and carbonates, and as such it is absorbed readily from the stomach as well as the intestines.

Tests for Arsenic

(i) *Sublimation Test*.—If As_2O_3 is given with food, water, milk, tea, etc., a portion, if it was administered as a fine powder, is likely to float on the surface, and if administered as a coarse powder it may be found as a sediment, and might therefore be detected in the vessel in which it was stored or administered. A careful inspection of the vessel, the food residue or the mucous membrane of the stomach may reveal the presence of fine or gritty particles of white or yellow arsenic adhering to them. They should be picked up, washed with water, dried and submitted to the sublimation test when the characteristic crystals of As_2O_3 (Fig. 44, p. 424) will be found on the cooler part of the tube.

(ii) *Reinsch's Test*.—It is of special value in forensic analysis as it may be applied to the tissues, stomach contents, urine and fæces without previous destruction of the organic matrix. The test is rapid and quite delicate requiring no special apparatus nor any special skill on the part of the analyst. This is carried out in the following way:

Take the material in a porcelain basin (about 200—300 c.c. capacity). If the material for analysis is solid, e.g., tissues, fæces or foodstuffs, add sufficient measured quantity of water and one-sixth volume of pure (arsenic-free) conc. HCl. In the case of urine, stomach washings, etc., dilution with water is not necessary. Suspend a strip of bright, arsenic-free copper foil (about 3" by $\frac{1}{4}$ ") in the porcelain basin as described on p. 423 and allow the mixture to boil gently. If arsenic is present as arsenious compounds, a steel-grey stain or a black deposit will be formed on the copper foil at once or in a few minutes. If no stain or deposit is noticed or if the result is doubtful, cool the mixture and add a few crystals of KI or ferrous sulphate or some sodium sulphite, mix thoroughly and boil again. This process will reduce the pentavalent arsenic compounds (arsenates) which do not readily produce the stain, to trivalent arsenious compounds and the characteristic stain of arsenic will appear in a few minutes. If no stain appears, continue boiling for about an hour, the volume of the liquid and the concentration of the acid being maintained by the addition of water or dil. HCl from time to time. If the copper foil remains bright, i.e.,

if the test is negative, it may be concluded that arsenic is absent in the material or possibly present in a very small quantity in the form of an organic arsenic compound which requires destruction of its organic matrix for liberation of arsenic. If, on the other hand, the copper foil shows a stain, it may be due to arsenic, antimony or bismuth or to sulphur compounds and occasionally to carbonaceous matter.

Now, take out the stained copper foil, wash with distilled water (taking care that the deposit, if any, is not detached and lost in washing), and then with alcohol and ether to remove particles of fat adhering to it. Wipe it dry by gently pressing it between the folds of a filter paper, cut into small pieces with a pair of clean scissors and then introduce the pieces into a flat or ordinary sublimation tube. Gently warm the lower end of the tube (about one inch) and then heat the pieces of copper, holding the tube in a slanting position on a small Bunsen flame—arsenic will be oxidized into As_2O_3 and deposited as a white sublimate on the cooler portion of the tube. *The preliminary warming of the tube as described above helps to form larger crystals which are easily detected* and not mistaken for anything else. Examine under the low power of the microscope when beautiful octahedral and monoclinic crystals of As_2O_3 (see fig. 44) will be found.

The Reinsch test detects 0.0001 grain or about 0.006 mgm. of arsenic. The negative result can, therefore, be safely interpreted as to indicate the absence of arsenic in a suspected case of arsenic poisoning because the material employed in the test will, almost certainly, contain much more than 0.006 mgm. of arsenic if the case be actually one of arsenic poisoning. The tables II and V (pp. 562, 564) will give an idea of the amounts of arsenic likely to be found in tissues in acute arsenic poisoning.

(iii) *Marsh's Test or Marsh-Berzelius Test.*—See page 556.

(iv) *Gutzeit's Test.*—See page 559.

(v) *Bettendorff's Test.*—This is an important test for arsenic. The importance lies in the fact that it can detect arsenic in the presence of antimony. The reaction is based

on the property that inorganic arsenic compounds are reduced by stannous chloride in presence of an excess of strong HCl to black arsenic. The test detects both arsenious and arsenic compounds and also differentiates inorganic from organic arsenicals. *Bettendorff's reagent* (modified by Winkler) is prepared by dissolving 1 part of crystallized stannous chloride $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 10 parts of strongly fuming HCl (sp. gr. 1.19—1.20, i.e., 37 to 39 per cent). The solution may be slightly brownish (due to traces of arsenic in the acid) but becomes clear and colourless after standing for some time.

Procedure. (a) *For Inorganic Arsenicals.*—Take 2 c.c. of the acid solution of the oxidized material in a test tube, add 10 c.c. of the reagent and heat the mixture gradually to boiling. Allow to stand for 30 minutes—a brown, brownish black or black precipitate of metallic arsenic is formed. A better result is obtained if the material is oxidized by the Babo-Fresenius method.

(b) *For Organic Arsenicals.*—Take 3 c.c. of the reagent in a test tube and add 1 c.c. of the solution of the organic arsenical and warm; no brownish nor any black colour or precipitate is formed, but a lemon yellow precipitate or colour will be noticed.

Quantitative Determination of Arsenic

The determination of arsenic or for the matter of that of any metal present in tissues, foodstuffs, urine, fæces, etc., is carried out in two stages, viz., (i) the destruction of the organic matter and liberation of the metal as an inorganic compound, and (ii) the application of a suitable method for its actual determination.

In case arsenic is present in fairly large quantities as indicated by the Reinsch test, the ordinary gravimetric method in which arsenic is precipitated and weighed as arsenious sulphide may be chosen. If it is present in very small amounts, the method of choice should either be the Marsh-Berzelius or the Gutzeit.

(i) *Destruction of Organic Matter.*—Various methods have been recommended for destruction of organic matter. The Babo-Fresenius method is chosen by a large number

of workers in spite of its defects (see p. 421). The nitric-sulphuric acid method of Ramberg, slightly modified, is, however, considered most suitable for this purpose and gives uniformly good results in a much shorter time. This method has already been described on p. 421. The acid solution of arsenic obtained by this method contains arsenic in pentavalent form which should therefore be reduced to arsenious form by treatment with SO_2 , HI , etc. (see Reinsch test, p. 425) after dilution with water. The excess of SO_2 , iodine, etc., is then expelled by boiling for some time. The solution is filtered, washed and the filtrate with the washings is made up to a known volume, an aliquot portion of which is submitted to a suitable quantitative method for determination of arsenic.

Organic arsenical preparations such as salvarsan should also be oxidized by the above method for destruction of its organic components so that it may be tested both qualitatively and quantitatively.

(ii) Determination of Arsenic. (a) *Gravimetric Method.*—Take an aliquot portion of the acid solution or the whole amount if necessary and pass H_2S till saturation, a yellow precipitate of arsenic sulphide As_2S_3 is formed. Allow the precipitate to settle, filter through a dry, weighed Gooch crucible and wash with water. Pass H_2S again through the combined filtrate and washings and if any precipitate appears filter the solution again through the same filter, wash and dry the precipitate in an air oven at about 100°C to constant weight. Multiply the weight of As_2S_3 by the factor 0.8042 to obtain the weight of As_2O_3 present in the aliquot portion of the acid solution taken. From this, the amount of As_2O_3 present in the known weight of the viscera or other material taken for quantitative determination may be calculated.

(b) *Marsh-Berzelius Method.*—The underlying principle of this very delicate test is the production of arsine AsH_3 (or stibine SbH_3 in the case of antimony) by the action of nascent hydrogen on a soluble inorganic arsenic compound and its subsequent decomposition by heat into metallic

arsenic and hydrogen, the former being deposited in the form of a grey mirror.

The apparatus in its simplest form consists of a flask provided with a tap funnel for generation of nascent hydrogen from pure zinc and pure H_2SO_4 , a drying tube charged with lead acetate cotton (absorbent cotton soaked in 10 per cent lead acetate solution and dried) in its first

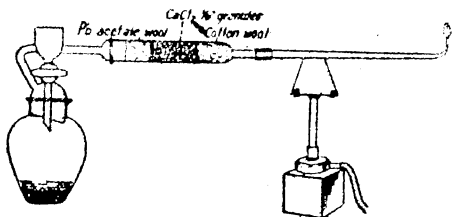


Fig. 60

half and with granular anhydrous calcium chloride in its second half, a special hard glass exit tube drawn out to a jet with its tip turned up and a small Bunsen burner. In the expensive form of this apparatus, the flask is replaced by an electrolytic cell where hydrogen is produced by electrolysis of dilute sulphuric acid.

Procedure.—Blank Experiment.—Take about 15 g. of pure granulated zinc in the flask, wash it out with water acidulated with HCl . add 15 c.c. of 10% solution of pure cadmium sulphate and allow to act for 15 minutes. The object of adding cadmium sulphate is to sensitize the zinc which in its purest form does not readily react with acids (Chaston Chapman). Pour dilute sulphuric acid (1 : 6) or dilute HCl (50 per cent) into the flask from the tap funnel (see Fig. 60). Allow the reaction to continue till the apparatus becomes completely free from air which is driven out with the stream of hydrogen in about 5-7 minutes. Kindle the gas at the jet, it burns with a small steady flame of hydrogen. Heat a portion of the exit tube with the Bunsen flame for about 15 minutes, no stain appears on the tube beyond the heated portion if the reagents are all pure and free from arsenic and antimony. If any stain appears, throw out the contents of the flask, wash with water several times, change the zinc and other reagents if necessary and repeat the experiment to obtain a satisfactory stainless blank.

At this stage the *qualitative Marsh test* may be performed (to form an idea of the amount of arsenic present in the

solution) by introducing through the tap funnel a few c.c. of the acid solution of the oxidized material containing arsenic and igniting the gas at the jet when a lilac-coloured or bluish-white flame appears at once. Heat the exit tube as described above, when a grey mirror of arsenic is formed at the cooler portion of the tube. For better result, particularly in quantitative work, a portion of the tube immediately beyond the heated portion is cooled with a small piece of filter paper soaked in water and kept cool by a drip-flow of water. This acts as a condenser and helps to obtain the arsenic mirror in a clean and compact form with a sharp margin. Instead of heating the exit tube if a cold porcelain capsule is depressed on the flame at the jet, arsenic is deposited on the porcelain as a shining steel-grey spot which is identified by the following tests:

- (1) It is readily soluble in sodium hypochlorite or bleaching powder solution (forming arsenates) while the antimony spots produced under the same condition of the experiment is not soluble.
- (2) If the spot is moistened with yellow ammonium sulphide and then evaporated by gentle heating, a bright yellow stain of As_2S_3 is left which is soluble in ammonia but insoluble in HCl, while the antimony spot gives an orange coloured stain of Sb_2S_3 , which is insoluble in ammonia but soluble in HCl.
- (3) If it is moistened with a concentrated solution of tartaric acid, it does not dissolve but the antimony spot readily dissolves forming $(SbO)_2C_4H_4O_6$.
- (4) If the spot is dissolved in a little HNO_3 and dried by gentle heating a whitish residue is left, which becomes red if touched with a drop of $AgNO_3$ solution if the stain be arsenical, while no change of colour is noticed in the case of antimony stain.

Now proceed with the actual determination. Begin a fresh experiment and place the Bunsen burner at the right place to heat the exit tube and then add a known amount of the acid solution, little by little, through the tap funnel until a shining arsenic mirror of suitable size has been formed in the tube. Allow this reaction to

continue, for about an hour or more so that the whole of the arsenic has been used up and the maximum intensity of the stain has been produced. Now heat the exit tube at a point about an inch proximal to the stain, draw it out and seal it. Seal also the distal end of the tube in the same way. The arsenic mirror thus produced in the tube is now ready for matching against standard arsenic tubes or mirrors, as they are called, prepared in the same way from the standard arsenic solution containing known quantity of arsenious oxide. The arsenic contents of these standard mirrors usually vary from 1/200 to 1/50 mgm. but mirrors having much smaller quantities of arsenic may also be prepared according to one's requirement.

Precautions about the Test.—(i) If the evolution of hydrogen is too brisk some of the arsenic may escape without being decomposed into arsenic and hydrogen. The action may, however, be controlled by putting the flask in an ice bath.

(ii) Rapid evolution of hydrogen and consequent production of heat may reduce a portion of the sulphuric acid to sulphurous acid or even to H_2S which interferes with the test.

(iii) The acid solution for this test should be free from chlorine, nitric or nitrous acid or salts of silver and mercury (particularly $HgCl_2$) otherwise the arsenic will be decomposed in the flask and the formation of the mirror will be retarded.

(iv) The use of HCl in the Marsh apparatus is, according to certain workers, likely to produce a zinc mirror and thus vitiate the result. The zinc mirror is unlike As or Sb readily soluble in dilute acids (Arbuckle and Thies, *Am. Chem. Abst.*, 1928).

(c) *Gutzeit Method.*—The original method has lately been considerably modified by different workers and the modification adopted by the British Pharmacopœia for determination of arsenic in drugs is also useful in toxicological investigation.

The underlying principle of this test is the production of arsine (as in Marsh's test) and development of a yellow or brown stain by the action of arsine on silver nitrate paper. Silver nitrate originally recommended by Gutzeit has now been replaced by mercuric chloride or still better by mercuric bromide, both of which produce a stable stain not affected by light or water as is the case with silver nitrate.

The *Gutzeit test* is also both qualitative and quantitative. It is quite simple and at the same time sensitive and reliable. The apparatus (see Fig. 61) consists of a wide mouthed bottle (about 120 c.c. capacity) fitted with a rubber bung through which passes a glass tube 200 m.m. in length and 6.5 m.m. in internal diameter. The lower end of the tube

is drawn out to a diameter of about 1 m.m. and a narrow hole (about 2 m.m. in diameter) is blown on the side. The upper end is provided with an ebonite disc (25 m.m. in diameter) having a central hole of 6.5 m.m. corresponding exactly to the diameter of the tube. The mercuric chloride paper (prepared by soaking a Swedish filter paper in a saturated soln. of HgCl_2 and dried at about 60°C in the dark) is placed on the ebonite disc and covered with a similar disc (C) having an identical central hole and kept in position by two spring clamps (B in Fig. 61). The HgCl_2 paper behaves like a diaphragm between the

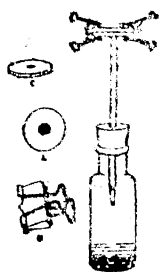


Fig. 61

holes of the two discs through which the gases pass. A piece of dry lead acetate paper (prepared by soaking filter paper in 10 per cent lead acetate solution) about 100 m.m. in width rolled into a coil, is inserted in the tube to hold back any H_2S likely to be formed there (not shown in the Fig.).

Procedure—Qualitative Test.—First perform a blank experiment with about 15 g. of arsenic-free zinc, 15 c.c. of dilute H_2SO_4 and 30 c.c. of water. Hydrogen passes through the tube containing the lead acetate paper and then through the mercuric chloride paper and a yellowish stain will appear on the mercuric chloride paper if any arsenic is present as an impurity in the reagents employed, and a brown or black stain on the lead acetate paper if any sulphide is present. If this blank experiment proves satisfactory add a few c.c. of the acid solution, prepared by destroying the organic matter as stated before, a yellow, brown or dark brown round stain (A) is formed in a few minutes or at once depending upon the amount of arsenic originally present in the material. This forms the qualitative test.

If the stain is faint, it may be intensified by keeping the paper in dilute HCl (1:1) for two minutes at a temperature of 50° to 60°C , washing in distilled water and drying, or better in normal ammonium hydroxide solution for about 5 minutes, and then washing and drying, the ammonia changing the yellow stain to dense coal-black colour.

Quantitative Determination

Repeat the experiment as before and add a measured amount (an aliquot portion of the total amount) of the acid solution. If the stain obtained by the qualitative test appears to be too deep, a smaller quantity of the solution has to be added. Allow the reaction to continue for about 40 minutes. If the evolution of hydrogen is not brisk (which may be due to the high degree of purity of the zinc used), put the generating bottle in a warm bath which will accelerate the reaction, or if the reaction is too brisk, place it in an ice bath to obtain a steady evolution of the gas. The stain thus obtained may be developed further by treating with ammonia and then matched against standard stains prepared exactly in the same way in another set of apparatus by taking different amounts of the standard solution.

Preparation of Standard Solution of Arsenic.—The standard solution is prepared by dissolving 100 mgm. of chemically pure and dry As_2O_3 in about 250 c.c. of dilute solution of pure Na_2CO_3 . This alkaline solution of As_2O_3 is acidified with arsenic-free dil. H_2SO_4 and made up to one litre with distilled water. 10 c.c. of this solution contains 1 mgm. of As_2O_3 . The standard solution (strong) keeps well indefinitely. It is further diluted by taking 10 c.c. and making up to one litre. 1 c.c. of this diluted standard solution contains 0.001 mgm. of As_2O_3 .

N.B. The diluted standard solution does not keep well and should, therefore, be prepared at frequent intervals or better at the time of actual determinations.

Determination of Arsenic in Earth.—The earth soaked with vomit, faeces, urine, etc., may be examined in the following manner: Prepare the sample carefully by mixing it thoroughly in a mortar and weigh out 25-50 g. in a beaker, dilute with water and render it alkaline if necessary with dil. NaOH solution. Heat it on a water bath for 2 hours with frequent shaking and leave it at room temperature for 24 hours. Filter through cotton wool and sand, wash, concentrate the filtrate with the washings to a small bulk and filter again through a filter paper. Evaporate to dryness on the water bath. Dissolve the residue in dil. arsenic-free HCl , add little bromine-water and heat on the water bath to destroy the organic matter. Remove the excess of bromine by heating, add some stannous chloride solution to reduce the arsenic to arsenious compounds and make up to a definite volume. Take an aliquot portion for determination of arsenic by any one of the methods described before.

Arsenic Contents of Normal Tissues, Excreta and Food.—As arsenic is widely distributed in nature, exact information about the amount of arsenic normally present in human tissues, excreta, foodstuffs, etc., is of much importance in toxicological investigation. When arsenic is present in large quantities in the viscera or urine or stomach contents of a suspected case of acute arsenic poisoning, no difficulty arises in giving an opinion as to the cause of death but if

only a few milligrams are found as in cases of chronic poisoning or slow arsenic poisoning, the question of normal arsenic content of the viscera, hair, urine, fæces, etc., comes in, that is to say, the investigation centres round the question—how much of the total amount of arsenic is of extraneous origin and how much normally occurs in the tissues or in the urine? The arsenic content of hair, particularly of the hair of women, is of much medicolegal significance in cases of 'slow poisoning'. If such hair is divided into three segments and each segment is analyzed separately, the arsenic contents of the individual segments will be found, to vary according to the period of ingestion of arsenic, that is to say, the segment yielding the maximum amount of arsenic would correspond to the period when arsenic was being ingested by the victim. The following tables would give an idea of the amount of arsenic present in common foodstuffs and in the normal tissues and excreta of Indians as also in cases of poisoning. This investigation was carried out by Bagchi & Ganguly and the results were published in the *Indian Medical Gazette* in 1937 & 1941 from which the tables have been taken.

TABLE I

Arsenic-contents of normal tissues. Figures indicate mgm. of As_2O_3 per kilo of fresh tissues or parts per million.

Tissues		Minimum	Maximum	Average
Liver	...	1.8	2.5	2.05
Kidney	...	0.75	1.0	0.82
Stomach	...	0.07	0.5	0.34
Spleen	...	Nil.	0.06	0.024
Small Intestine	...	0.25	0.5	0.41
Heart	...	Nil.	0.05	0.03
Blood	...	Nil.	0.07	—
Lungs	...	Nil.	Nil.	—
Brain	...	—	0.04	—
Bone	...	1.6	2.12	1.83
Tooth	...	1.6	2.0	1.76
Hair	...	1.2	1.62	1.29
Skin	...	1.25	1.75	1.50
Thyroid	...	1.25	1.50	1.3
Mammary gland	...	1.5	2.5	1.76
Testis	...	Nil.	Nil.	—
Ovary	...	0.05	0.12	0.085
Uterus	...	Nil.	Nil.	—
Placenta	...	0.75	1.1	0.95

TABLE II

Arsenic-contents of tissues in suicidal and homicidal cases. Mgm. of As_2O_3 per kilo.

Tissues	Case No. 1	Case No. 2	Case No. 3	Case No. 4
	Suicide	Suicide	Homicide	Homicide
Liver ...	279.3	667.3	333.0	372.0
Kidney ...	188.7	589.4	217.0	238.8
Stomach ...	60.0	903.7	412.0	403.0
Lungs ...	11.6	28.0	37.0
Spleen ...	6.7	15.0	17.0

TABLE III

Arsenic-contents of normal urine and fæces. Mgm. of As_2O_3 per litre or kilo.

	Minimum	Maximum
Urine ...	0.004	0.075
Fæces ...	0.17	0.50

TABLE IV

Arsenic-contents of some common foodstuffs from the Calcutta market. Milligrams of As_2O_3 per kilo of fresh materials.

Mutton ...	0.33	Potato, patol, green	
Beef ...	0.43	plantain, pumpkin,	
Chicken ...	0.14	jhinga, green papaya,	
Rohit fish		lady's finger, carrot,	
(Labia Rohita)	0.36—0.66	beet ...	Nil.
Lobster (edible		Cabbage ...	0.12
portion)	1.42—1.70	Cauliflower ...	0.036
Hilsa fish ...	3.0	Spinach ...	0.03
Sea fish		Rice ...	0.2
Pomfret ...	3.13	Dals (5 kinds)	0.1—0.26
Sole ...	2.6	Wheat flour	0.06
Plaice ...	3.2	Orange, guava, mango,	
		tomato, tamarind ...	Nil.
Banana (Martaban)	0.04	Grapes ...	0.006
Apple (imported) with		Bread ...	0.06
skin ...	0.01	Cake ...	0.12
Raisin, pistachio, almond,		Rasagolla ...	0.08
walnut, ground		Sandesh ...	0.08
nut	0.11—0.54	Sea water (from Puri)	0.38
Cocoonut ...	0.036	Calcutta tap water	0.004
		Aerated water (soda)	0.008

TABLE IV, *Arsenic-contents (Continued)*

Cow milk ...	0.11	Biscuit ...	0.05
Buffalo milk ...	0.12	Sugar (Indian) ...	0.05
Human milk ...	0.048	Glucose (imported) ...	0.008
Condensed milk ...	0.19	Baby food (imported) ...	0.02
Ghee ...	0.09	Common salt (Indian) ...	0.06
Mustard oil ...	0.12	Red pepper ...	0.02
Hydrogenated fat ...	0.06	Ginger, turmeric ...	Nil.

Organic Arsenicals.—Quite a large number of trivalent organic compounds or arsphenamines, such as salvarsan, sulpharsenol, etc., and similar pentavalent compounds such as soamin or atoxyl, stovarsol, carbarson, etc., have been synthesized for the treatment of syphilis and other diseases. They are extensively used in medicine and many cases of severe arsenic poisoning have been known to occur some of which having ended fatally. They have thus introduced new problems in toxicological analysis.

The arsenic-contents of the various organic arsenicals vary in different compounds. For example, salvarsan contains 29.30 per cent. of arsenic (although theoretically it should contain 33.6 per cent), neosalvarsan about 20 per cent, carbarson about 29 per cent, stovarsol about 27 per cent, atoxyl about 25 per cent (theoretical value being 31.3 per cent in anhydrous samples) and sulpharsenol about 20 per cent of arsenic.

Atoxyl and a few other pentavalent compounds are eliminated quickly and completely through the kidneys while salvarsan and neosalvarsan and possibly the other arsphenamines are excreted slowly although they appear in the urine just a few minutes after their administration by the intravenous route. After 6 hours, salvarsan or neosalvarsan ceases to appear in the urine (Autenrieth) although the elimination of the arsenic through the kidneys (possibly in ionizable form) continues for weeks. It is stated that about 14 per cent of the arsenic derived from salvarsan is eliminated in the urine in 14 days but its excretion through the faeces may be much greater than that through the kidneys, as much as 54 per cent of the arsenic may be eliminated through the faeces in one week. In fatal cases of salvarsan poisoning, appreciable quantities of arsenic are found in the tissues

sometimes several weeks after the last injection. The table given below (Table V) gives an idea of the amount of arsenic usually found in tissues in such cases of poisoning and thereby indicates the slow process of elimination of organic arsenicals from the human system. A dose of 0.9 g. of neosalvarsan would introduce into the system about 0.18 g. or 2.7 grains of As (equivalent to about 3.5 grains of As_2O_3) which is much higher than the minimum fatal dose (2 grains) of As_2O_3 , but as it is in organic form its toxicity is considerably less than that of soluble inorganic arsenicals and therefore no poisoning occurs. In certain cases symptoms of poisoning develop and end in fatal results, possibly due to partial decomposition of the drug into some toxic products.

TABLE V

Arsenic-contents of tissues in poisoning by neosalvarsan and other organic arsenicals. Figures indicate milligrams of As_2O_3 per kilo of fresh tissues. Taken from the records of the Chemical Examiner, Bengal.

Case I				Case II			
An Anglo-Indian girl, aged 19, died two weeks after the last injection in a course of neosalvarsan. Committed suicide by taking oxalic acid. No indication of arsenic poisoning before death.				A Bengalee Hindu girl, aged 19, died six days after the second injection of 2 c.c. of solusalvarsan. Developed arsenic encephalitis on the third day. Brain not received for analysis.			
Liver	Kidney	Stomach	Intestine	Liver	Kidney	Stomach	Intestine
13.0	6.4	0.87	1.0	3.3	3.0	0.7	—

Detection of Salvarsan in the Urine.—Take 8 c.c. of the urine in a test tube, acidify with 6-8 drops of HCl, cool in ice water, add 3-4 drops of 0.5 per cent solution of NaNO_2 . Pour gently a few c.c. of this mixture on 5-6 c.c. of a 10 per cent colourless solution of resorcinol made alkaline with 2-3 c.c. of 20 per cent solution of Na_2CO_3 . Allow to stand for sometime, a dark red zone appears in the upper liquid or on mixing both the solutions, a red colour develops.

Atoxy! urine gives a purplish-red or orange colour if tested by the above method and normal urine gives a yellow colour.

Detection of Salvarsan in the Tissues.—Mix the finely minced tissue with water, acidify with tartaric or hydrochloric acid and evaporate to dryness. Extract this residue 2 or 3 times with hot 90 per cent alcohol on a water bath, filter and evaporate the filtrate to a syrupy residue. To this residue add an excess of absolute alcohol, a few c.c. at a time and stirring with a glass rod to mix them thoroughly. The alcohol takes up the arsenical and precipitates the tissue material. Filter and evaporate the alcoholic extract to dryness. Take up the residue with warm water and examine the aqueous solution by Bettendorff's reagent (see p. 555) and also by the test (diazotization and coupling with resorcinol) described in the preceding paragraphs.

TABLE VI

Tests for Differentiating Arsenious from Arsenic Compounds.

Reagents	Arsenious compounds, * Arsenites, e.g., K_3AsO_3 , As_2O_3	Arsenic Compounds, Arsenates, e.g., K_3AsO_4 , As_2O_5
Conc. HCl and H ₂ S.	A yellow precipitate of As_2S_3 soluble in caustic alkalis, ammonia, Am_2S , etc., producing thio-arsenites: $As_2S_3 + 3(NH_4)_2S = 2(NH_4)_3AsS_2$ $As_2S_3 + 6KOH = K_3AsS_3 + K_3AsO_3 + 3H_2O$	No precipitate until As (ic) is reduced to As (ous) by passing H_2S through boiling solution for a longer period. During reduction sulphur is precipitated. $2As_2O_5 + H_2S = As_2O_3 + 4H_2O + 4S$
$AgNO_3$ (in neutral solution).	A yellow precipitate of silver arsenite Ag_3AsO_3 , soluble in ammonia and dilute acids.	A reddish brown or brickred precipitate of silver arsenate Ag_3AsO_4 , soluble in NH_4OH and dilute acids.
$CuSO_4$ (in neutral solution).	A bright green precipitate of cupric arsenite $CuHAsO_3$ (Scheele's green).	A pale blue precipitate of cupric arsenate $CuHAsO_4$.

TABLE VI—Continued.

Reagents.	Arsenious compounds, Arsenites, <i>e.g.</i> , K_3AsO_3 , As_2O_3	Arsenic Compounds, Arsenates, <i>e.g.</i> , K_3AsO_4 , As_2O_5
Magnesia mixture (containing $MgSO_4$ or $MgCl_2$ with excess of NH_4Cl and ammonia).	No precipitate.	A white crystalline precipitate of $Mg(NH_4)AsO_4$: $K_3AsO_4 + MgCl_2 + NH_4Cl = Mg(NH_4)AsO_4 + 3KCl$.
Solution of Iodine in KI.	The colour of iodine solution is discharged due to oxidation of arsenious acid by iodine and reduction of iodine to colourless HI: $As_2O_3 + 2I_2 + 2H_2O = As_2O_5 + 4HI$.	No change of colour.

5. Mercury

Mercury forms two series of compounds—mercurous and mercuric. The mercurous compounds such as calomel Hg_2Cl_2 are insoluble and less toxic and the mercuric compounds such as corrosive sublimate $HgCl_2$ are readily soluble and, therefore, more toxic.

Mercurial poisoning occurs from its ingestion by the mouth, from inhalation of mercury vapour through the lungs, from absorption of the metallic mercury and its compounds through the unbroken skin, wounds and mucous surfaces and from subcutaneous and other forms of injections.

In this country, the mercuric salts, particularly the corrosive sublimate, account for most cases of acute mercury poisoning which prove fatal. The metallic mercury (in liquid form) is frequently administered with food by village people with criminal intention but so far no poisoning has been known to occur in such cases, partly because of the fact that the metal not being miscible with food or drink is usually left out in the vessel and partly due to the amount swallowed being not sufficient to produce symptoms of poisoning.

Chronic mercurial poisoning which is mostly of industrial or occupational origin is still unknown in this country.

Tests for Mercury

Reinsch's Test.—This test detects mercury in mercuric, mercurous or in metallic form. The first and the last give an immediate deposit on copper but the mercurous compounds require prolonged boiling. As in the case of arsenic, this test is also very useful for detection of mercury compounds in tissues, urine, faeces, foods, etc. The procedure for carrying out the test is the same as described under arsenic (p. 553). The deposit of mercury in the copper foil, instead of being steel-grey or black as in arsenic, is beautiful silvery white which gives a sublimate of the characteristic globules of mercury.

In case the material for examination is scanty and the globules of mercury obtained in the sublimation tube are few and not very decisive, the presence of mercury may be confirmed by the following tests: Take out the bits of copper foil from the sublimation tube by gentle shaking, introduce a small crystal of iodine into the tube and keep it plugged with cotton wool for sometime, the white sublimate of mercury now becomes red due to formation of red iodide of mercury HgI_2 , best seen under the low power of the microscope.

The Reinsch test does not work properly if nitrates, chlorates and other oxidizing substances are present in the material under examination and steps should therefore be taken to remove them as described on p. 425.

The positive Reinsch test does not indicate the form in which mercury was originally present in the material examined as both metallic mercury and calomel as well as mercuric salts give a positive test. To express an opinion if a mercuric salt was administered, it would be necessary to repeat the test in the following manner: Filter the material (stomach contents, stomach washings, remnants from the cup or other vessels, etc.) after dilution with water if necessary, and submit the filtrate to this test. If again positive,

it will indicate that a soluble compound of mercury present in the filtrate is responsible for the second positive test. Now to a portion of the filtrate add a few drops of dilute KI solution when a red precipitate of mercuric iodine HgI_2 is formed which is soluble in excess of KI solution. The presence of a mercuric salt is thus confirmed (see table on p. 571).

Quantitative Determination of Mercury

As in the case of arsenic, the determination of the quantity of mercury in any material involves two stages, viz., the destruction of the organic matrix for liberation of the metal and the application of a suitable method for actual determination of the inorganic compound thus formed.

The nitric-sulphuric acid method of destruction of organic matter as described under arsenic is not at all suitable for mercury which is almost completely lost by volatilization.

The Babo-Fresenius method (slightly modified) is considered most suitable for liberation of mercury although there is a possibility of some loss of mercury by volatilization. It is best carried out in the following manner: Weigh or measure out a definite amount of the material and take in a litre flask fitted with a reflux condenser. In the case of viscera or other solid material add sufficient water to make it gruel-like in consistency, then add about $1/3$ of its volume of chemically pure conc. HCl and a few grams of solid $KClO_3$ and mix the contents carefully by shaking. Heat the mixture over a wire gauze on a small Bunsen flame or on a boiling water bath. From time to time add a small amount of $KClO_3$ and shake the flask. The compressed $KClO_3$ tablets are much better than and preferable to powdered $KClO_3$, because the latter is quickly decomposed by hot acid with rapid evolution of chlorine while the former gives a slow but steady evolution of Cl gas. Continue heating as before until the contents of the flask become a uniform, straw coloured liquid free from organic matter, except some fatty substances in suspension which cannot be further oxidized. If heating for about an hour after the last addition of $KClO_3$ produces no darkening of the mixture, the oxidation of organic matters may be taken as completed. It takes 4—6 hours to attain this stage. Filter and wash. Add to the filtrate and the washings sufficient sodium sulphite or bisulphite to reduce the excess of chlorine into hydrochloric acid. Warm the liquid on the water bath and pass a current of air to expel the excess of SO_2 . The solution is now ready for actual determination which is carried out as follows:

(1) Pass pure H_2S gas (purified by passing the ordinary gas through a tube containing anhydrous calcium chloride and then through a tube containing asbestos fibres charged with fine crystals of iodine and finally through another tube containing KI solution)

through the solution for at least half an hour. Filter through a weighed Gooch and wash the precipitate with water. Pass purified H_2S through the combined filtrates and washings. Filter it again through the same filter. Wash and dry to constant weight. The weight of HgS represents the amount of mercury present in the known amount of the material. Multiply the weight of HgS by the factor 0.862 or 1.167 to obtain the weight of metallic mercury or mercuric chloride respectively.

(2) *Alkaline Digestion Method of Naidu.* This method, worked out by Naidu and co-workers (Jour. & Proc. Inst. Chem., 1943), has been found very useful and may be carried out without any loss of mercury. The method is based on the facts that (i) animal tissues are completely dissolved by strong solution of caustic alkalis, (ii) mercuric sulphide is soluble in an aqueous solution of sodium sulphide and caustic soda, and (iii) it is insoluble in an aqueous solution of sodium bicarbonate saturated with H_2S .

Procedure.—Weigh out 25–50 gm. of finely minced tissues or measure out a definite volume (as large a quantity as available but concentrated to a syrupy consistency) of urine or stomach washings. Add sufficient 30 per cent $NaOH$ solution (about 10 c.c. for every 30 g. of solid material) and excess of sodium sulphide solution (10 per cent $NaOH$ solution saturated with H_2S , about 10 c.c. for every 30 gm.) to keep the mercuric sulphide dissolved in the solution. Metallic mercury will not be affected and will remain as such. Calomel Hg_2Cl_2 will be converted into HgS ($Hg_2Cl_2 + Na_2S = HgS + Hg + 2NaCl$). HgS will dissolve rapidly in a mixture of Na_2S and $NaOH$ ($HgS + Na_2S = Na_2HgS_2$, a soluble compound).

Heat the mixture on a boiling water bath for about 2 hours till it becomes pasty. Dilute with 200–300 c.c. of water and continue heating till the organic matter has all gone into solution. Cool, acidify with conc. HCl , add solid $NaHCO_3$ till faintly alkaline and saturate with H_2S . HgS is completely precipitated (in the absence of $NaOH$, Na_2S being no longer formed). The precipitate consists of HgS if $HgCl_2$ was present, $HgS + Hg$ if calomel was present or Hg alone if metallic Hg was present in the material. Filter, wash and transfer the filter paper with the precipitate into a beaker. Acidify with dilute HCl and saturate with H_2S (iron being eliminated at this stage). Filter, wash the precipitate, transfer the filter paper to a beaker, add excess of bromine water and leave overnight. HgS dissolves in bromine water as $HgBr_2$, and metallic Hg also becomes $HgBr_2$. Filter and wash the residue which consists of sulphur (and some brominated fatty acids). Add 0.5 g. of KCl to the combined filtrates and washings and boil to drive off the bromine. Acidify the debrominated solution with dilute HCl and saturate with H_2S . Filter through a weighed Gooch, wash the precipitate of HgS , dry in a steam oven and weigh to constant weight. Multiply the weight of HgS by the factor 1.167 or 1.015 or 0.862 to obtain the weight of $HgCl_2$, Hg_2Cl_2 , or metallic mercury respectively.

TABLE VII

Tests for Differentiating Mercurous from Mercuric Compounds

Reagents	Mercurous compounds, e.g., $\text{Hg}_2(\text{NO}_3)_2$	Mercuric compounds, e.g., HgCl_2
HCl or chlorides.	A white precipitate of Hg_2Cl_2 , which is insoluble in acids and is turned black by alkalis — with caustic alkali black mercurous oxide Hg_2O , and with ammonia a black compound $\text{Hg}_2\text{NH}_2\text{Cl}$ ($\text{HgNH}_2\text{Cl} + \text{Hg}$) is formed.	No precipitate.
H_2S .	A black precipitate of mercuric (not mercurous) sulphide HgS and metallic Hg is formed. HgS soluble in <i>aqua regia</i> and in conc. solution of Na_2S . $\text{HgS} + \text{Na}_2\text{S} = \text{Na}_2\text{HgS}_2$. Presence of free alkali and polysulphides helps to dissolve HgS quickly.	At first a white precipitate of $\text{HgCl}_2 \cdot 2\text{HgS}$ is formed which on passing more H_2S changes to yellow and orange and finally to black mercuric sulphide HgS (see mercurous).
NaOH or KOH .	A black precipitate of mercurous oxide Hg_2O [$\text{Hg}_2(\text{NO}_3)_2 + 2\text{NaOH} = \text{Hg}_2\text{O} + 2\text{NaNO}_3 + \text{H}_2\text{O}$].	A yellow or orange precipitate of mercuric oxide HgO ($\text{HgCl}_2 + 2\text{KOH} = \text{HgO} + 2\text{KCl} + \text{H}_2\text{O}$).
NH_4OH .	A black precipitate of $\text{HgNH}_2\text{NO}_3 + \text{Hg}$ is formed.	A white ppt. of amino-mercuric chloride HgNH_2Cl formed.
KI .	A yellowish green precipitate of mercurous iodide Hg_2I_2 , soluble in excess of the reagent; on heating a grey or greyish black precipitate is formed due to separation of Hg . $\text{Hg}_2\text{I}_2 + 2\text{KI} = \text{K}_2\text{HgI}_4 + \text{Hg}$	A yellow ppt. rapidly changing to red mercuric iodide HgI_2 , soluble in excess of either of the reagents and produces a colourless soln. with excess of KI . K_2HgI_4 is formed. Nessler's reagent is an alkaline soln. of this compound.

6. Antimony

Poisoning by antimony compounds was comparatively rare in this country but since the import and use of cheap enamelled ware in which antimony is used as an ingredient of its glaze, cases of acute antimony poisoning have been occasionally met with. The antimony oxide of the glaze is dissolved by tartaric or citric acid of *chutnies*, pickles, home made lemonades, etc., if they are prepared or stored in such enamelled vessels. The extensive and sometimes injudicious use of tartar emetic and other organic antimonials for the treatment of Kala-azar has also been a source of acute and chronic cases of poisoning some of which have proved fatal. Homicide or suicide by antimony compounds have not been reported so far.

Tests for Antimony

As signs and symptoms of acute antimony poisoning are almost identical with those of arsenic poisoning and as the chemical properties of antimony resemble greatly those of arsenic, the methods of detection and determination of antimony in viscera, etc., are also the same as in cases of arsenic poisoning.

(i) *Reinsch's Test*.—To carry out this test the destruction of organic matter is not usually necessary and the

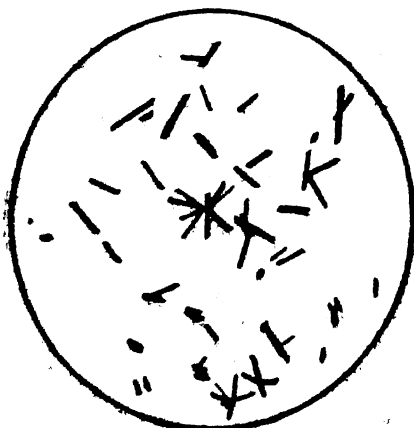


Fig. 62

1885) and Witthaus has also stated the same fact in his

material may be tested directly. Proceed in the same way as in arsenic (see p. 553). In the case of antimony either amorphous deposit or needle shaped crystals of Sb_2O_3 (instead of octahedral and monoclinic as in arsenic) will be found. Wormley claims to have found octahedral crystals of Sb_2O_3 (*Micro-chemistry of Poisons*,

book but it has not been confirmed by later workers so far. We too have not been able to find any octahedral crystal Sb_2O_3 in hundreds of experiments carried out with pure antimony compounds by the students of our college while doing their practical work in toxicological chemistry. In most cases the needle shaped crystals and in few only amorphous deposits are formed.

(ii) *Marsh's Test*.—For this and other tests described below, the destruction of organic matter is necessary and which should be done by the nitric-sulphuric acid method described on p. 421. The other details of the test are exactly the same as in arsenic (see p. 556). The identification of the antimony spot obtained on the porcelain capsule and its differentiation from arsenic have been described on p. 558.

(iii) *Gutzeit's Test*.—The details of this test have been given on p. 559. The antimony stain on the mercuric chloride paper cannot, however, be differentiated from the similar stain obtained in the case of arsenic.

(iv) *Other Chemical Tests*.—They are applicable only when larger amounts of antimony salts are available. The following tests are helpful:

(a) H_2S produces in an acid solution of antimonious compounds an orange precipitate of Sb_2S_3 which is soluble in NaOH or ammonium sulphide. In antimonie compounds H_2S gives a mixture of Sb_2S_3 , Sb_2S_5 and sulphur.

(b) HCl dissolves antimony compounds and yields a solution of SbCl_3 which if poured into water gives a white precipitate of antimonious oxychloride SbOCl . On adding a drop of very dilute HCl , the precipitate redissolves quickly but on further dilution with water it reappears. The oxychloride is soluble in tartaric acid and tartrates but the corresponding bismuth salt is insoluble.

Quantitative Determination of Antimony

The acid solution prepared from a known quantity of the material by destroying the organic matter by the nitric-sulphuric acid method and then treating with a suitable reducing agent should be used for determining the quantity of antimony.

(i) The gravimetric method by saturating the acid solution with H_2S to obtain Sb_2S_3 and then weighing the sulphide as in the case of arsenic (p. 556) is not suitable in cases of antimony poisoning, as the quantity of antimony likely to be found in such cases is too small for direct weighing. It is, however, useful if large quantity of antimony happens to be present in the material for examination.

(ii) *Gutzeit Method*.—Dilute the acid solution (oxidation product) with water to make it up to a known volume. Take an aliquot portion and perform the Gutzeit test. Treat the stained mercuric chloride paper with a normal solution of ammonia for five minutes, wash and dry as described under arsenic. The brownish stain turns black. Match the black stain against the standard stains obtained with different amounts of the standard solution of antimony and calculate the result accordingly.

(iii) *Volumetric Method*.—This method is most suitable for organic antimonials. Take 0.1 g. of the substance in a Kjeldahl flask, add 5 c.c. of conc. H_2SO_4 , 3.3 g. of K_2SO_4 and one-third of a 9 c.m. filter paper. Heat until the solution becomes colourless. Cool and dilute with 150 c.c. of water. Add 7 c.c. conc. HCl and boil for 2 minutes. Cool to room temperature and then titrate with $\text{N}/10$ KMnO_4 solution to pink colour. 1 c.c. of $\text{N}/10$ KMnO_4 is equivalent to 0.006 g. of antimony (*vide Jour. & Proc. Inst. Chem.*, p. 86, 1945).

Preparation of Standard Solution of Antimony

Dissolve 2.306 g. of pure tartar emetic in a litre of water. This solution contains 1 milligram of antimonious oxide per c.c. Take 10 c.c. of this solution and dilute to one litre. This diluted standard solution contains 0.01 mgm. of Sb_2O_3 per c.c. This solution or a more diluted one, if necessary, should be freshly prepared within a few hours of use.

Organic Antimonials

Quite a large number of organic preparations of antimony have lately been introduced for the treatment of Kala-azar. The most important of these antimonials are the pentavalent compounds, e.g., ureastibamine (Brahmachari), stibosan and neostibosan (von Heyden), etc. The injudicious use of these drugs has resulted in fatal antimony poisoning.

Antimony is stored in the liver and other organs just as is the case with arsenic. It is eliminated mostly through the kidneys and to a certain extent through the gastrointestinal tract. It appears in the urine within 5-15 minutes after the intake.

Its detection and determination in the urine, faeces, tissues, etc., are carried out by one of the methods described above.

Antimony contents of viscera of cases of kala-azar who died after injections of organic antimonials. Figures indicate mgm. per kilo of fresh tissues.

Cases	Liver	Kidney	Stomach	Remarks
H.M. aged 23. Died 4½ hrs. after 2nd inj. urea stibamine	12.5	4.5	1.0	0.1 gm. in all in 4 days. Last dose 0.05 gm. Vomiting and purging.
M.M. aged 15. Died 5 days after 2nd inj. urea stibamine	19.0	9.0	...	0.1 gm. in all in 10 days. Last dose 0.05 gm. Nephritis.
M.M. aged 22 ...	7.5	4.0	0.8
M.M. aged 14 ...	8.1	3.5	0.7
H.M. aged 28. Died 5 mts. after 5th inj. urea stib. ...	5.0	4.0	0.3	0.35 gm. in all in 3 weeks. Last dose 0.05 gm.

7. Barium

Poisoning by barium salts is not very common and the cases so far investigated in Bengal are all accidental due to negligence of the pharmacist in serving the doctor's prescription. Barium sulphide is usually mistaken for barium sulphate which is used by radiologists in preparing 'barium meal' for X-ray examination of the alimentary tract. Lately a fatal case of barium poisoning occurred in Calcutta in which the victim was given a dose of four drams of barium chloride instead of magnesium sulphate prescribed by the doctor and the patient died in about 3 hours.

Extraction of Barium.—Barium may be readily extracted from the viscera, urine, stomach-contents, fæces, etc., in the following way: Evaporate the urine, fæces, stomach washings, etc., to dryness and transfer the residue to a platinum or nickel crucible and ash in the usual way. Fuse the ash with sodium carbonate to convert barium sulphate, if any formed in the tissues, into barium carbonate. Cool and extract with dil. HCl. The acid extract is tested for barium.

Tests for Barium

(i) Add a few drops of dil. H_2SO_4 to the acid solution, a white precipitate of BaSO_4 is formed which is insoluble in HNO_3 and HCl .

(ii) Neutralize the acid solution with ammonia and add a few drops of neutral potassium chromate solution, a yellow precipitate of barium chromate BaCrO_4 is formed which is soluble in HNO_3 and HCl but insoluble in acetic acid.

(iii) Neutralize the solution with sodium carbonate or faintly acidulate with acetic acid, add a few drops of 5 per cent solution of the sodium salt of chloronitrotoluenesulphonic acid, a crystalline precipitate is formed even in 1 in 2,000 parts. It gives no precipitate with calcium or strontium.

(iv) *Feigl's Spot Test*.—Soak a piece of filter paper in a freshly prepared solution of sodium rhodizonate and allow it to dry, touch it with a drop of a neutral solution of a barium salt, a reddish stain or precipitate is formed. Moisten the stain with dil. HCl , the red colour changes to scarlet.

Quantitative Determination of Barium

Weigh or measure out a definite quantity of the material, render alkaline with NaOH solution and ash in a platinum capsule. Dissolve the ash in a little dilute HCl with heat if necessary, dilute with water, filter and wash the filter with water till it is acid free. Combine the filtrate and the washings and keep them carefully and mark (A). Transfer the filter paper to the platinum capsule, dry and incinerate. Add some solid Na_2CO_3 and heat to fusion. Cool the fused mass extract with dil. HCl , filter and wash with hot water. Combine this filtrate and the washings with the portion marked (A). To the mixture add dil. H_2SO_4 to precipitate barium as BaSO_4 . Wash, dry and weigh the precipitate. The amount of BaCl_2 or BaS present in the weighed quantity of the material may then be calculated. The weight of BaSO_4 multiplied by 0.8922 or 0.72258 gives the weight of BaCl_2 or BaS respectively.

8. Lead

Lead and lead poisoning were known in ancient times. Hippocrates (about 400 B.C.) described the illness caused by working in the smelting of lead ores.

The metallic lead and all lead compounds are poisonous. They are introduced into the system though oral ingestion, through inhalation as fine dust, and through the unbroken skin. The respiratory route is more effective in producing lead intoxication than the other routes, and in fact most cases of poisoning of industrial origin are traced to this route. The basic carbonate $2\text{PbCO}_3 \cdot \text{Pb(OH)}_2$ (white lead), the sub-oxide (Pb_2O) and other oxides of lead such as PbO (litharge) and Pb_3O_4 (red lead) are insoluble compounds but they are more dangerous than many soluble lead compounds. These compounds of lead are relatively more soluble in blood serum than in water. The magnitude of harm caused by these compounds is therefore more pronounced in those industries in which the above oxides are used. The following table (Table I) extracted from Jacob's Poisons, Hazards and Solvents (1941) shows the relative solubility of these compounds in water and blood serum:

TABLE I

*Relative Solubility of some Lead Compounds in Water and Serum.
Figures indicate milligrams of lead compounds per litre at 25°C*

Lead Compounds	In Blood Serum	In Water
Lead monoxide, PbO (litharge) ...	1152.0	17.1
Lead carbonate PbCO_3 ...	33.3	1.7
Lead sulphate ...	43.7	44.0
Lead (metallic) ...	578.0	—

Chronic lead poisoning is almost entirely of industrial origin. Such cases were practically unknown in this country a decade or two ago but with gradual industrialization cases of chronic lead poisoning have been steadily increasing particularly in industrial centres like Calcutta and Bombay.

The custom of applying Chinese vermilion (powdered cinnabar) to the hair parting by married Hindu women has been prevailing in this country, particularly in Bengal, from very ancient times. The extensive use of cheap vermilion manufactured from red lead has lately been found to be the cause of an insidious type of lead poisoning among Hindu women, the absorption of lead taking place through the

scalp where the vermilion is applied and also through inhalation especially of the portion spilt during its use.

Acute lead poisoning is mostly accidental and occasionally suicidal, but rarely homicidal. As lead compounds are potent abortifacients, they are frequently used in procuring criminal abortion. The village quacks use red lead and litharge for treatment of various diseases including common cold and thus cause accidental death to their patients.

Lead tetraethyl.—The *leaded motor spirit*, that is petrol mixed with lead tetraethyl $\text{Pb}(\text{C}_2\text{H}_5)_4$ (about 1 part in 1,300 parts of petrol by volume) now extensively used in this country as a motor fuel, is likely to be a potent source of lead poisoning among those who would handle this lead compound carelessly.

Lead tetraethyl is a heavy volatile and colourless liquid (sp. gr. 1.62 at 15°C , b.p. 200°C with decomposition). It is insoluble in water and ether but soluble in alcohol, acetone, petrol and other organic solvents and mixes with fats and oils in all proportions. It is a lipid solvent and possesses slight solvent action on rubber. It decomposes when evaporated or exposed to sunlight with the formation of a poisonous compound, lead triethyl hydroxide $\text{Pb}(\text{C}_2\text{H}_5)_3\text{OH}$ and other products.

Lead tetraethyl being a lipid solvent and volatile compound is readily absorbed through the skin and the respiratory tract and as such cases of *acute poisoning* have been known to occur among the workers in petrol depots in the West. Lately three fatal cases (one from Chittagong and 2 from Calcutta) of lead tetraethyl poisoning have been investigated in the Chemical Examiner's department, Calcutta. These men along with several others were engaged in cleaning large empty petrol reservoirs in which leaded petrol was stored. There was some 'scum' or semi-solid substance at the bottom of the tanks which these men were cleaning, and after a few days they developed signs and symptoms indicating lesions in the Central Nervous System, e.g., headache, insomnia, restlessness, forgetfulness, delusion, delirium, &c. and " signs of madness such as shouting, knocking the head

against the wall', etc., as described by the Medical Officer of the Company. In the autopsy of one of the victims the "right heart was found partially filled with *bright red fluid blood*" suggestive of carbon monoxide poisoning.

It is interesting to note that similar cases described as *lead tetraethyl poisoning* have been recorded by the League of Nations in its publication "Occupation and Health" (1934). These cases developed "insomnia with restless, excited dreams, talkativeness and delusion, vertigo and headache" and some became "violently maniacal—shouting, leaping from the bed, smashing furniture", etc. In two fatal cases the temperature "rose up to 110°F just before death" and in another fatal case the blood "failed entirely to coagulate and it had the colour typical of carbon monoxide hæmoglobin".

The viscera of our cases showed appreciably high lead contents (vide Table II) but not so high as to suggest *acute lead poisoning* as met with in toxicological practice (vide Table V). These are, therefore, the cases of acute lead tetraethyl poisoning and not ordinary lead poisoning, acute or chronic. The poisonous compound, lead triethyl, the decomposition product of lead tetraethyl, is obviously responsible for rapid involvement of the Central Nervous System and the production of the characteristic syndrome. The difference in the concentration of lead triethyl or other poisonous decomposition products is likely to explain the difference in signs and symptoms in individual cases and in their post-mortem findings.

TABLE II

Lead contents of viscera of three suspected cases of acute lead tetraethyl poisoning. Figures indicate milligrams of lead (as Pb) per kilo, or parts per million, of fresh tissues.

Tissues		Chittagong Case	Calcutta Case No. 1	Calcutta Case No. 2
Liver	...	2.5	3.2	2.8
Kidney	...	2.1	2.0	2.0
Stomach	...	1.6	1.8	1.6

Selection of Materials for Detection of Lead.—As lead is excreted mostly in the fæces and less in the urine, the examination of the former gives a definite indication of the amount of absorption of lead. In suspected chronic poisoning both fæces and urine should be examined. The route by which lead is introduced into the system gives an idea of the distribution of lead in the various organs of the body. If it is absorbed from the gastro-intestinal tract, most of the lead is likely to be found in the liver. If the absorption takes place through the respiratory tract, the lungs, kidneys and also the brain are likely to contain much larger quantities than in the other organs. The bones are regarded as the lead depots of our system where lead is stored as insoluble lead phosphate. Administration of heavy doses of acids such as phosphoric acid, or alkalies such as sodium bicarbonate, producing acidosis or alkalosis as the case may be, converts the insoluble lead phosphate either into a soluble lead salt or a colloidal lead phosphate which is then thrown into the general circulation for elimination with the urine and fæces. Lead is also found in large amount in the hair which may be regarded as its dumping ground. As hair is a dead tissue, the amount of lead deposited in hair cannot be thrown back into the general circulation and is thus eliminated from the system. In this respect hair may also be regarded as an eliminatory organ for the waste and toxic products of the system. The largest amount of lead and in fact of all metals present in the human system, is found in the black hair which is, therefore, a very useful material for information regarding the absorption and retention of lead in chronic lead poisoning (Mineral Constituents of Human Hair, Bagchi & Ganguly, *Ann. Biochem. & Expt. Med.*, 1941).

Tests for Lead

After destruction of organic matter by the nitric-sulphuric acid method and removal of the excess of H_2SO_4 by heat, the acid solution containing some of the lead as insoluble PbSO_4 , is heated with a slight excess of saturated solutions of ammonium acetate and ammonium citrate and

then neutralized with ammonia. Diluted with water and tested for lead by the following tests:

(i) Acidify with HCl and pass H_2S through the acid solution, a dark brown or black precipitate of lead sulphide is formed which is soluble in dilute HNO_3 . On boiling with conc. HNO_3 it is oxidized into lead sulphate.

(ii) Add a few drops of KI , a yellow precipitate of PbI_2 is formed which dissolves on heating and reappears on cooling in the form of beautiful golden yellow spangles.

(iii) Add a few drops of K_2CrO_4 solution, a yellow precipitate of lead chromate PbCrO_4 is formed which is soluble in HNO_3 .

(iv) *Spot Test*.—Take a drop of the solution in a filter paper and add to it a drop each of a 1 per cent solution of pyridine in water and of a mixture of 0.1 per cent gallo-cyanine and sodium bicarbonate, a deep violet colour develops if lead is present.

Lead contents of Normal Tissues. Excreta and

It has been definitely established that lead is a normal constituent of human tissues and is excreted in appreciable amounts in normal urine and faeces and that the source of lead is the food we take. The investigations carried out here to determine the lead contents of the urine, faeces, tissues, etc. of Indians have shown that the results obtained by the European and American workers are rather too high and cannot be regarded as standards for comparison in the case of an Indian suspected to have been suffering from or to have died of lead poisoning. The following results worked out by Bagchi, Ganguly and Sardar and extracted from *Ind. Jour. Med. Res.*, 1939, and *Ann. Biochem. & Expt. Med.*, 1940, would give an idea of the lead contents of human tissues, excreta, foodstuffs, etc. The normal tissues required in this investigation were all collected from cases in which death was due to street accidents, throttling, drowning, hanging, etc., and not to any illness causing starvation and thereby a disturbed metabolism.

TABLE III

Lead contents of normal tissues. Figures indicate milligrams of lead (as Pb) per kilo or parts per million, of fresh tissues.

Tissues		Minimum		Maximum		Average	
Liver	0.31	...	0.82	...	0.57
Kidney	0.37	...	0.71	...	0.50
Spleen	0.30	...	0.52	...	0.36
Stomach	0.20	...	0.60	...	0.41
Small intestine	0.20	...	0.60	...	0.38
Heart	0.45	...	0.75	...	0.56
Lung	0.30	...	0.60	...	0.45
Blood	0.11	...	0.45	...	0.24
Brain	Nil.	...	0.10	...	0.073
Testis	0.30	...	0.40	...	0.34
Uterus	0.05	...	0.47	...	0.28
Ovary	Nil.	...	Nil.	...	Nil.
Muscle	0.14	...	0.70	...	0.33
Skin	0.33	...	0.50	...	0.44
Scalp	1.0	...	1.2	...	1.1
Fat	Nil.	...	Nil.	...	Nil.
Bone	6.8	...	39.3	...	15.8
Tooth	15.5	...	23.0	...	20.7
Nails	11.3	...	12.7	...	20.0
Hair	16.0	...	508.0	...	80.9

TABLE IV

Comparative statement showing lead contents of tissues in different nationalities. Figures indicate milligrams of Pb per kilo of fresh tissues.

Tissue	Indian		American		British	
	Bagchi et al.	1939	Kehoe et al.	1933	Tompsett and Anderson,	1935
Liver	...	0.82	...	0.80	...	4.63
Kidney	...	0.71	...	0.70	...	2.60
Heart	...	0.75	...	Trace	...	—
Lung	...	0.60	...	0.30	...	0.88
Intestines	...	0.68	...	0.20	...	—
Spleen	...	0.52	...	Trace	...	5.9
Scalp	...	1.20	...	1.30	...	—
Brain	...	0.10	...	0.10	...	0.72
Bone	...	8.5(rib)	...	11.4(long bone)	...	12.9(rib)

TABLE V

Lead contents of tissues of two cases of acute poisoning by red lead investigated in the Chemical Examiner's Department, Calcutta. In milligrams per kilo.

Tissues			Case No. 1		Case No. 2
Liver	17.3	...	13.8
Kidney	4.6	...	2.8
Stomach	3.4	...	1.46
Spleen	2.1	...	—
Small intestine	—	...	1.33

TABLE VI

Lead contents of tissues of three suspected cases of chronic lead poisoning—death due to other causes. In milligrams per kilo.

Tissues	Case No. 1 Death due to burns.		Case No. 2 Death due to frac- ture of skull.		Case No. 3 Death due to injury.
Liver	... 0.98	...	3.60	...	1.5
Kidney	... 4.0	...	3.9	...	—
Lungs	... 0.60	...	1.09	...	3.6
Spleen	... 0.72	...	1.87	...	—
Small intestine	... 1.50	...	0.9	...	1.2
Brain	... 0.60	...	0.75	...	—

TABLE VII

Lead-contents of normal urine and fæces of Indians. In milligrams per litre or kilo.

		Hindus		Muslims		Anglo- Indians
Urine	{ Minimum	... Nil.	...	Nil.	...	0.024
	{ Maximum	... 0.016	...	0.026	...	0.040
	{ Average	... 0.008	...	0.014	...	0.031
Fæces	{ Minimum	... 0.08	...	0.10	...	0.13
	{ Maximum	... 0.14	...	0.16	...	0.18
	{ Average	... 0.11	...	0.13	...	0.15

TABLE VIII

Comparative statement showing lead-contents of normal urine in different nationalities. In milligrams per litre of urine.

	Minimum	Maximum	Average	Investigators
German	0.01	0.55	—	Litzner & Weyrauch (1935)
American	0.04	0.08	0.05	Kehoe et al (1935)
British	Nil.	0.133	0.04	Francis, Harvey & Buchan (1929)
Australian	0.02	0.050	0.04	Cooksey & Walton (1929)
Indian (including Anglo-Indian)	Nil.	0.040	0.018	Bagchi, Ganguly & Sardar (1939)

TABLE IX

Lead-contents of hair in different nationalities. In milligrams per kilo of hair. Average figures given.

Nationality	Men	Women
Europeans resident in Calcutta	20.8	18.4
Indians	28.0	114.5
Bengali Hindu	26.7	180.9
Bengalee Muslim	42.4	50.4
Punjabi	20.2	45.5
Madras	22.7	—
U. P. & Behari	21.6	—
Other provincials (Parsee, Oriya, Marwari, etc.)	20.0	26.3

TABLE X

Lead-contents of hair of different shades of colour. In milligrams per kilo of hair.

Colour of hair	Minimum	Maximum
Deep black (Bengalee women)	170.0	508.0
Brown, auburn and other shades (Europeans)	9.0	16.5
Grey, containing 0 to 25 per cent of black or brown (European and Indian)	3.0	21.0

Quantitative Determination of Lead (Method of Lynch, Slater & Osler)

(1) *For minute quantities of lead*, weigh out 50-100 grams of viscera or other solid material or measure 250-500 c.c. of the urine or other liquid material and evaporate to a small bulk. Oxidize the organic matter by the nitric-sulphuric acid method (see pp. 421-22). To the acid solution (oxidation product) in a beaker add 5 c.c. each of 10 per cent solutions of ammonium acetate and ammonium citrate and render the mixture alkaline with ammonia. Add 2 c.c. of 5 per cent solution of NaCN, transfer to a 150 c.c. separating funnel and make up the volume to about 50 c.c. Extract immediately with three portions of 0.1 per cent chloroformic solution of diphenylthiocarbazone (10, 7.5 and 5 c.c.) shaking for 2-3 minutes, after which extract 2 to 3 times with chloroform only (using 15, 10, 5 c.c., etc.) until the last traces of the dye are completely removed from the aqueous mixture. Wash the combined extracts with water in another separating funnel and run off into a large Pyrex tube (8×1 in.) containing a small crystal of K_2SO_4 and distil to drive off the chloroform. Oxidize the residue in the Pyrex tube by heating with 1 c.c. of conc. HNO_3 in a boiling water bath for 30 minutes, after which add 0.5 c.c. of conc. H_2SO_4 and heat over a small flame adding conc. HNO_3 drop by drop as required a few drops of 5 per cent solution of pure $CuSO_4$ may be added as a catalyst to hasten the oxidation. When oxidation is complete, boil off the remaining HNO_3 , adding a few c.c. of water if necessary. After cooling, add about 20 c.c. of water and transfer to a 50 c.c. Nessler cylinder. Set up two or three similar Nessler cylinders and measure in from a burette different amounts of the standard solution of lead (containing 0.01 mgm. of lead per c.c.), e.g., 1, 2, and 3 c.c. or larger quantities according to the amount of lead likely to be present in the unknown solution and dilute with water. Add 5 c.c. of 6N (approx.) ammonia to each cylinder to render its contents alkaline. Then add 5 c.c. of ammonium acetate solution and 2 c.c. of sodium cyanide solution to each cylinder and make up the volume to 50 c.c. with distilled water and finally add to each 2 drops of 4 per cent sodium sulphide solution and mix them up intimately with a glass rod, a pale brown colour develops. The colour of the unknown solution is matched against the standard solutions in the usual way of colorimetric determination. Calculate the result accordingly (Analyst, 1934).

(2) *For relatively large quantities of lead*, heat the oxidized material (acid solution) to expel the excess of H_2SO_4 as indicated by appearance of crystals in the beaker (should not be heated beyond this stage). Cool, add 10 c.c. of water, cool again and then add drop by drop ammonia to make it almost neutral (slightly acid to litmus). Transfer to a 100 c.c. flask, rinse with water and make up to 100 c.c. mark—a slight precipitate of silica may be noticed which is negligible. Take an aliquot portion (10 c.c. or more according to the amount of lead likely to be present) in a 50 c.c. Nessler tube, add 2 c.c. ammonium citrate solution (50 per cent) or 1 g. solid ammonium citrate to keep down the phosphates (it also makes the

colour stable), 1 c.c. of 10 per cent solution of KCN or 0.1 g. of solid KCN to hold iron in solution as $K_4Fe(CN)_6$, and a measured quantity of ammonia (about 2 c.c.) to render it alkaline. At this stage, precipitation of phosphates may take place if they are in excess. If so, add a few drops of dil. HCl to dissolve the precipitate, more ammonium citrate (2-3 c.c. or more) and again render alkaline with ammonia. Make up the volume to the 50 c.c. mark, when a yellow colour (due to iron) develops (A).

In three other Nessler tubes, take measured quantities of the standard lead solution (say, 3, 4 & 5 c.c.) and to each of them add 15 c.c. H_2O , 2 c.c. ammonium citrate solution, 1 c.c. KCN solution, 2 c.c. ammonia, and make up the volume to 50 c.c. mark, no yellow colour develops as there is no iron in these tubes. With glass rod add one or two drops or more of a very dilute solution of $FeCl_3$ to each of these tubes just to match the yellow colour with that of the unknown solution in (A).

Now add 2 drops of 10 per cent solution of sodium sulphide to each of the four Nessler tubes and thoroughly mix with a glass rod, a yellow colour develops. Match the colour of the unknown against the closest standard and calculate the amount of lead.

In this method of determination, a blank experiment should always be carried out. If a yellow colour appears in this Nessler tube (due to iron as an impurity in the reagents), a trace of $FeCl_3$ is to be added to the Nessler tube containing the standard with which it is to be compared. Then after adding sodium sulphide solution, the two Nessler tubes are matched and the amount of lead present in the reagents is thus found out. The blank figure is to be deducted from the value obtained in the unknown solution.

Preparation of Standard Solution of Lead.—0.16 g. of lead nitrate accurately weighed and dissolved in about 20 c.c. of water. Add 50 c.c. strong HNO_3 , mix and cool. Make up to 1000 c.c. with water (1 c.c. of this = 0.1 mgm. of lead). 10 c.c. of this solution accurately measured in a standard burette and diluted to 100 c.c. gives the diluted standard solution (1 c.c. = 0.01 mgm.).

9. Copper

Fatal cases of copper poisoning frequently occur in Bengal and also in other parts of India. They are mostly suicidal or accidental and rarely homicidal. Copper sulphate is also taken as an abortifacient but the woman usually dies of copper poisoning 3 or 4 days after the miscarriage.

Detection of Copper.—Copper is widely distributed in nature. It is found in the soil, in natural waters, and in most of our food materials and it is present in appreciable quantities in different tissues of the animal body. It fact, it is one of the important constituents of the hæmoglobin of

blood and the physician nowadays prescribe copper for regeneration of blood in anæmia. In these circumstances, mere detection of a trace of copper in the tissues, urine, stomach contents, etc., is of no toxicological significance unless its quantity is determined. If it is present in large quantities along with the characteristic blue stains on the mucous membrane of the stomach no ambiguity arises and the common chemical tests for copper can be successfully tried. In such cases the stomach contents and the washings as well as the vomit can be tested directly without destroying the organic matter. If the tests given below fail to detect any copper the materials should be oxidized by the nitric-sulphuric acid method as described before and tested again. The urine and tissues should first be oxidized and the tests to be applied to the acid solution.

Tests for Copper

(i) In some clear filtrate of the stomach contents, vomit, etc., slightly acidulated with HCl, put in a polished iron needle and keep it for some time or overnight, when metallic copper will be deposited on the needle.

(ii) To some clear filtrate or to the oxidation product add a few drops of ammonia, a bluish green precipitate of a basic salt $\text{CuSO}_4\cdot\text{Cu}(\text{OH})_2$ is formed which readily dissolves in excess of ammonia and a blue colour develops.

(iii) To the solution (neutral or faintly acid) add a few drops of $\text{K}_4\text{Fe}(\text{CN})_6$ solution, a reddish brown colouration or precipitate of cupric ferrocyanide $\text{Cu}_2\text{Fe}(\text{CN})_6$, soluble in warm dilute HNO_3 , is formed.

(iv) *Feigl's Test*.—To the solution (neutral or faintly acid), add a few drops of dil. zinc nitrate solution and 1-2 c.c. of *Feigl's reagent* (prepared by dissolving 8 g. of mercuric chloride and 9 g. of ammonium thiocyanate in 100 c.c. of distilled water), a pink, purple or deep violet colour or precipitate is produced if copper is present.

(v) Render the solution just alkaline with ammonia. If iron is present a precipitate of ferric hydroxide is formed which is removed by filtration. To the filtrate add a few drops of 0.1 per cent solution of sodium diethyl-dithiocarbamate. a brown colour develops at once. As this test is very

sensitive, a highly diluted solution of copper is to be employed.

Quantitative Determination of Copper

Weigh out 25—50 g. of the finely minced tissue or the stomach contents or measure out as much urine or other liquid matter as available and oxidize by the $\text{HNO}_3 + \text{H}_2\text{SO}_4$ method. The acid solution is now ready for quantitative determination by one of the following methods:

(1) *Volumetric method for relatively large quantities of copper.*—Take an aliquot portion of the acid solution or the whole of it if the amount of copper is expected to be very small, and heat to boiling. Pass H_2S to saturation. Allow the precipitate to settle. Filter and wash with H_2S water. Transfer the filter with the precipitate to a 100 c.c. conical flask, add 5 c.c. each of concentrated H_2SO_4 and conc. HNO_3 and heat till the brown nitric fumes disappear and white fumes of SO_3 appear. If the solution is not colourless, add a few drops of HNO_3 from time to time and continue heating till the solution becomes colourless or faintly straw coloured and white fumes appear. Cool, dilute with about 25 c.c. of water, add an excess of bromine water and boil till the solution becomes free from bromine. Cool, add some strong ammonia, mix and boil until the excess of ammonia is driven off. Add 2-3 c.c. of glacial acetic acid and boil for a minute. Cool and add 10 c.c. of 30 per cent KI solution and titrate at once with N/10 sodium thiosulphate solution with starch solution as the indicator. One c.c. of N/10 $\text{Na}_2\text{S}_2\text{O}_3$ is equivalent to 0.0063 g. of Cu or 0.02497 g. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

(2) *Colorimetric method for small quantities of Copper in Viscera, Stomach-contents, Faeces, Urine, etc. (Tompsett's method—slightly modified).*—Oxidize the material by the nitric-sulphuric acid method and remove traces of HNO_3 by ammonium oxalate solution and of excess of H_2SO_4 by heating on a sand bath. If any white insoluble matter is present, dilute the digest to 20 c.c. with water, add 1 c.c. conc. HCl and boil until solution is complete. Cool and make up to a known volume. Take an aliquot portion (10 c.c. or less depending upon the amount of Cu likely to be present, about 0.01—0.03 mgm. of Cu being the most suitable concentration for matching), add 5 c.c. of 20 per cent sodium citrate solution and 6 c.c. of 4 per cent sodium pyrophosphate solution (2-3 c.c. for urine, 4-5 c.c. for faeces, foodstuffs, vomit, etc., and 6-10 c.c. for viscera—amounts depending upon the iron-content of the material).

Render the mixture alkaline with ammonia, add 5 c.c. of amyl alcohol (iso-amyl alcohol gives better result) and 5 c.c. of 0.2 per cent aqueous solution of sodium diethyl-dithiocarbamate (2 per cent solution recommended by Tompsett is difficult to prepare as the dye is not readily soluble in water) and shake well. Separate the amyl alcohol layer, filter it and match the colour of amyl alcohol against that of a standard solution treated exactly in the same way. If a colorimeter is used, the standard solution containing 0.01 mgm. of copper is quite suitable. If matching is done in Nessler cylinders, 2

or 3 standards should be set up. Small Nessler cylinders (15-20 c.c. capacity) may be specially made for this purpose. N.B.—*This method works best if the amount of copper does not exceed 0.1 mgm.*

Preparation of Standard Solution of Copper.—It is prepared by dissolving 0.3928 g. of pure crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in a litre of water. This forms the stock solution, 1 c.c. of which = 0.1 mgm. of copper. The dilute standard solution (1 c.c. = 0.01 mgm. of Cu) is prepared by diluting stock solution 10 times with water at the time of determination.

Copper-Contents of Normal Tissues.—The copper-contents of normal tissues of healthy individuals have been determined and shown in Table I. The tissues for this investigation were collected from police morgues of Calcutta and from cases in which death was due to street accidents, stabbing, throttling, gunshot wounds, etc., and not from hospital cases in which illness and starvation are likely to disturb the metabolism of the metal. The results obtained in these cases show fairly well marked difference from those obtained by the workers in the West, and as such any opinion based on the findings of those workers are likely to be erroneous in a case of poisoning in this country.

TABLE I

Copper-contents of normal human tissues. Figures indicate milligrams of Cu per kilo of fresh materials.

Tissues	Minimum	Maximum	Average	Tissues	Minimum	Maximum	Average
Liver ...	4.2	30.0	15.2	Hair ...	18.0	155.0	52.0
Kidney ...	10.0	17.0	12.5	Muscles ...	2.4	4.0	3.3
Spleen ...	4.2	8.3	5.7	Thyroid ...	4.0	7.0	5.7
Heart ...	5.0	9.5	7.2	Pancreas ...	6.0	7.0	6.5
Blood ...	4.0	6.0	5.0	Uterus ...	2.5	6.4	5.0
Brain ...	6.5	8.0	6.9	Ovary ...	3.7	5.0	4.1
Stomach ...	3.7	8.5	5.2	Testis ...	2.5	4.0	3.3
Small intestine	5.2	8.0	6.5	Placenta ...	8.75	13.75	—
Large intestine	7.5	17.0	11.5	Fetal liver ...	35.0	37.5	—
Bone ...	4.0	13.0	7.1	Fetal kidney ...	10.0	12.5	—
Skin ...	4.0	4.6	4.3				

In cases of acute copper poisoning, death is not rapid and the victim survives 3 or 4 days or even more and as such quite a large proportion of the ingested metal is eliminated from the system, and the tissues give comparatively low figures for copper (cf. arsenic) as shown in Table II.

As copper plays an important role in the formation of blood, the presence of quite a large amount of this metal in the tissues of fetuses and new-born babies is significant (see Table).

TABLE II

*Copper-contents of tissues in cases of suicidal copper poisoning.
In milligrams per kilo of fresh materials.*

			Minimum		Maximum		Average
Liver	34.0	...	150.0	...	74.0
Kidney	30.0	...	260.0	...	72.0
Stomach	8.0	...	90.0	...	27.0

10. Manganese

Acute poisoning by manganese salts is comparatively rare and is caused by ingestion of potassium permanganate. It is either accidental or suicidal, no fatal cases being yet recorded. Death from chronic manganese poisoning is, however, frequently met with among workers in manganese mines owing to inhalation of dust but such cases are yet unknown in this country.

Detection of Manganese.—As cases of manganese poisoning do not usually prove fatal the materials received for analysis are the stomach washings, vomit, urine and faeces. In cases of poisoning by opium and other organic poisons, the washings of the stomach with weak permanganate lotion are frequently sent for analysis and the presence of mangnaese in this material may be mistaken for manganese poisoning in the absence of a correct history of the cases which is rather the rule than an exception in this country.

Simple detection of manganese in viscera is not sufficient for expression of a positive opinion as manganese has

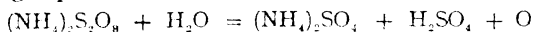
been proved to be an essential constituent of human tissues. A quantitative determination of manganese in the tissues is therefore necessary in fatal cases of manganese poisoning.

Tests for Manganese

(i) In stomach washings, manganese is usually found as a black precipitate of manganese hydroxide. Collect a small amount of this precipitate (some food materials being also present along with the hydroxide) and heat in a platinum capsule with a few drops of saturated caustic potash solution and a small crystal of KNO_3 , a green mass of potassium manganate K_2MnO_4 is formed. Cool, dilute with excess of water and acidify with dil. H_2SO_4 , the pink colour of KMnO_4 develops which is discharged by oxalic acid.

(ii) For urine and stomach washings having no solid residue, evaporate in a Pyrex beaker about 50 c.c. of the material to dryness, add a few c.c. H_2SO_4 (1:2) and 3-4 drops of conc. HNO_3 and heat until oxidation is complete. Continue heating till white fumes of SO_3 are given off, the chlorides which interfere with the test being expelled by volatilization during this stage. Take up the residue in water, add 2 or 3 drops of 2 per cent AgNO_3 solution (silver acts as a catalyst and also removes chlorides, if any, as AgCl) and filter. To the filtrate add excess of solid ammonium persulphate $(\text{NH}_4)_2\text{S}_2\text{O}_8$ and heat the mixture on a water bath for 15-20 minutes, when the pink colour of permanganate appears.

Ammonium persulphate oxidizes according to the following equation:



(iii) In the case of viscera, vomit and faeces, take 5-10 g. or more of the finely minced material and oxidize with conc. sulphuric and nitric acids and proceed as in (ii).

Quantitative Determination of Manganese

Take a known quantity of the material and ash in a large silver dish. Take up the ash in HCl and evaporate to dryness. (In the case of liquid samples, evaporate to dryness with conc. HCl). Add 2.5 c.c. of dil. H_2SO_4 (1:2) and 3-4 drops of conc. HNO_3 . Evaporate on a water bath and then on a sand bath to dryness and then gently ignite on Bunsen flame just to drive off the acid. Add again 2-3 c.c. of dil.

H_2SO_4 (1:2) and a little water and evaporate till the white fumes of SO_3 appear. At this stage all traces of chlorides disappear. Cool, dilute and add 2 c.c. of 2 per cent AgNO_3 solution, shake and filter into a 50 c.c. flask. Add slight excess of solid ammonium persulphate (in the proportion of about 2.5 grams of persulphate for each milligram of manganese likely to be present) and make up to 50 c.c. and then warm the solution on a water bath until the maximum depth of permanganate colour is developed which takes about 10-15 minutes. At the same time prepare standards by diluting 0.2, 0.4, 0.6 c.c., etc., of the standard manganous sulphate solution to about 50 c.c. and treat them exactly as the unknown sample was treated. Transfer the sample and the standards to Nessler tubes and compare the colours immediately. Calculate the result on the strength of the standard solution matched (1 c.c. of the standard solution = 0.1 mgm. of manganese).

Preparation of Standard Solution of Manganese.—Dissolve 0.2873 g. of KMnO_4 in about 100 c.c. of water. Acidify the solution with H_2SO_4 and heat to boiling. Add slowly a dilute solution of oxalic acid to discharge the colour of permanganate. Cool and dilute to one litre. One c.c. of the solution contains 0.1 mgm. of manganese.

II. Chromium

Acute poisoning by chromium is fairly common in other countries and is caused by ingestion of chromic acid, potassium chromate and potassium dichromate which are about 100 times more toxic than the salts in which chromium exists as kation, e.g., chromium chloride, chromium sulphate, etc. Fatal cases of chromium poisoning have also been recorded in this country. Most cases of poisoning are suicidal and accidental and only a few are homicidal. The dichromate has also been used in procuring abortion.

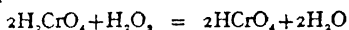
Detection of Chromium.—As chromium compounds are readily eliminated through the kidneys and bowels, the urine and faeces are suitable materials for examination even if death has been rapid. The liver and kidneys retain chromium in appreciable amounts and they also yield evidences of chromium poisoning. The stomach contents, vomit and the stomach washings may contain the unchanged chromates or dichromates and the filtrate which is yellow coloured may be tested directly for chromium. If the tests described below fail to detect chromium in the material the possibility of reduction of chromates into chromium salts (chromium as kation) which do not respond to these tests, and the presence of large quantities of organic matter which

mask the colour changes, are to be considered. The same difficulties will also arise in the case of urine and viscera in which chromium salts and not chromates are present. In such circumstances, the destruction of the organic matter and reconversion of chromium compounds into chromates are called for. The destruction of organic matter may be readily done either by direct incineration and then dissolving the ash in HCl, or by oxidation with HNO_3 and H_2SO_4 as described before. The acid solution will be green due to the presence of chromic chloride or sulphate. Evaporate the acid solution to dryness and mix the residue with an excess of a mixture of solid Na_2CO_3 and KNO_3 and fuse. The fused mass will now contain chromium as chromates. Extract the fused mass with warm water and filter, the filtrate becomes yellow which may be tested for chromates.

Tests for Chromium

(i) To the filtrate slightly acidified with dil. H_2SO_4 , add a few drops of KI solution, iodine is liberated indicating the presence of dichromate.

(ii) *Perchromic acid Test*.—To the filtrate acidified with dil. H_2SO_4 , add 3-4 c.c. of ether and then add drop by drop with continual shaking slight excess of hydrogen peroxide solution, the ether layer becomes blue due to formation of perchromic acid which is soluble in ether. This test indicates the presence of a chromate or a dichromate.



(iii) To the filtrate acidified with acetic acid, add a few drops of lead acetate solution, a yellow precipitate of lead chromate is formed which is soluble in HCl and in excess of NaOH solution but not in ammonia.

(iv) To the filtrate acidified with acetic acid, add a few drops of AgNO_3 solution, a red precipitate of silver chromate is formed which is soluble in HNO_3 and in NH_3 .

(v) *Carbazide Test*.—Acidify the filtrate with H_2SO_4 or acetic acid, take a drop of it on a porcelain plate and add to it a drop of the *carbazide reagent* (0.2 per cent solution of diphenyl carbazide in 10 per cent acetic acid or in a mixture of one part of glacial acetic acid and 9 parts of

alcohol), a beautiful pink or violet colour develops in the presence of chromates. This is a very delicate test.

(vi) Add to the filtrate a slight excess of conc. H_2SO_4 and then add a reducing agent, e.g., alcohol or formalin, the yellow colour of the solution changes to green chromium sulphate, i.e., from chromium anion to chromium kation.

Quantitative Determination of Chromium

Take up a known quantity (25-50 g.) of the material, destroy the organic matter as described before. Saturate the acid solution with H_2S in the manner discussed on p. 423. The acid filtrate (after separation of sulphides) containing chromium as $\text{Cr}_2(\text{SO}_4)_3$ is mixed with an excess of $\text{Na}_2\text{CO}_3 + \text{KNO}_3$ and evaporated to dryness. Fuse the dry residue with a little more of $\text{Na}_2\text{CO}_3 + \text{KNO}_3$. Extract the fused mass by heating with water, filter and make up the filtrate which contains chromium as K_2CrO_4 to a definite volume and take an aliquot portion for determination of the chromate.

Acidify the alkaline solution with acetic acid and add barium chloride solution, when a mixed precipitate of barium sulphate and barium chromate (yellow coloured) is formed. Filter the precipitate through a fine Swedish filter paper and wash the precipitate to free it from nitrates. Place the filter paper containing the precipitate in a beaker, add sufficient concentrated solution of Na_2CO_3 and boil, insoluble barium carbonate and soluble chromate are formed. Filter and wash. If the residue of barium carbonate is not quite white, treat it again with sodium carbonate in the same manner and filter. Combine the filtrates containing the chromate in solution, evaporate to a small bulk and transfer to a stoppered conical flask. Add 1-2 g. of KI and acidify with HCl. Allow the mixture to stand for 5-10 minutes and titrate with N/10 $\text{Na}_2\text{S}_2\text{O}_3$ solution. When the green colour of the reduced chromate begins to predominate over the brownish-red of the free iodine, add a few drops of freshly prepared starch solution and complete the titration until the blue colour of the starch is discharged. Take the reading and calculate the amount of chromium. 1 c.c. of the standard $\text{Na}_2\text{S}_2\text{O}_3$ is equivalent to 0.00173 g. of chromium or 0.049 g. of $\text{K}_2\text{Cr}_2\text{O}_7$ or 0.0033 g. of chromic anhydride (CrO_3).

INTERNATIONAL ATOMIC WEIGHTS (1941)

	Symbol	Atomic Weight O = 16		Symbol	Atomic Weight O = 16
Aluminium ...	Al	26.97	Neodymium ...	Nd	144.27
Antimony ...	Sb	121.76	Neon ...	Ne	20.183
Argon ...	A	39.944	Nickel ...	Ni	58.69
Arsenic ...	As	74.91	Niobium ...	Nb	92.91
Barium ...	Ba	137.36	Nitrogen ...	N	14.008
Beryllium ...	Be	9.02	Osmium ...	Os	190.2
Bismuth ...	Bi	209.00	Oxygen ...	O	16.000
Boron ...	B	10.82	Palladium ...	Pd	106.7
Bromine ...	Br	79.916	Phosphorus ...	P	30.98
Cadmium ...	Cd	112.41	Platinum ...	Pt	195.23
Caesium ...	Cs	132.91	Potassium ...	K	39.096
Calcium ...	Ca	40.08	Praseodymium	Pr	140.92.
Carbon ...	C	12.01	Protoactinium	Pa	231
Cerium ...	Ce	140.13	Radium ...	Ra	226.05
Chlorine ...	Cl	35.457	Radon (Niton)	Rn	222.0
Chromium ...	Cr	52.01	Rhenium ...	Re	186.31
Cobalt ...	Co	58.94	Rhodium ...	Rh	102.91
Columbium ...	Cb	92.91	Rubidium ...	Rb	85.48
Copper ...	Cu	63.57	Ruthenium ...	Ru	101.7
Dysprosium ...	Dy	162.46	Samarium ...	Sm	150.43
Erbium ...	Er	167.2	Scandium ...	Sc	45.10
Europium ...	Eu	152.0	Selenium ...	Se	78.96
Fluorine ...	F	19.00	Silicon ...	Si	28.06
Gadolinium ...	Gd	156.9	Silver ...	Ag	107.880
Gallium ...	Ga	69.72	Sodium ...	Na	22.997
Germanium ...	Ge	72.60	Strontium ...	Sr	87.63
Gold ...	Au	197.2	Sulphur ...	S	32.06
Hafnium ...	Hf	178.6	Tantalum ...	Ta	180.88
Helium ...	He	4.003	Tellurium ...	Te	127.61
Holmium ...	Ho	164.94	Terbium ...	Tb	159.2
Hydrogen ...	H	1.0080	Thallium ...	Tl	204.39
Indium ...	In	114.76	Thorium ...	Th	232.12
Iodine ...	I	126.92	Thulium ...	Tm	169.4
Iridium ...	Ir	193.1	Tin ...	Sn	118.70
Iron ...	Fe	55.85	Titanium ...	Ti	47.9
Krypton ...	Kr	83.7	Tungsten ...	W	183.92
Lanthanum ...	La	138.92	Uranium ...	U	238.07
Lead ...	Pb	207.21	Vanadium ...	V	50.95
Lithium ...	Li	6.940	Xenon ...	Xe	131.3
Lutecium ...	Lu	174.99	Ytterbium ...	Yb	173.04
Magnesium ...	Mg	24.32	Yttrium ...	Y	88.92
Manganese ...	Mn	54.93	Zinc ...	Zn	65.38
Mercury ...	Hg	200.61	Zirconium ...	Zr.	91.22
Molybdenum ...	Mo	95.95			

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